The Journal of
Clinical Endocrinology and Metabolism

VOLUME 21 APRIL, 1961 NUMBER 4
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SODIUM RETENTION WITH HUMAN GROWTH HORMONE AND ITS SUBFRACTIONS*†

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ABSTRACT

The administration of 10 mg. of human growth hormone within four hours produced a mean retention of approximately 30 mEq. of sodium and potassium per twenty-four hours in 3 of 5 normal subjects receiving a constant dietary intake; there was no change in urinary aldosterone. In 2 there was slight retention—without the rise in aldosterone that was found in a more prolonged study. Urinary sodium decreased after administration of human growth hormone in 2 patients with primary aldosteronism who had been refractory to daily intramuscular injections of 20 mg. of desoxycorticosterone acetate for four days. The magnitude of sodium retention was increased in primary aldosteronism, possibly because of the potassium-depleted state. Sodium retention was also observed in a bilaterally adrenalectomized subject after human growth hormone was administered. Results indicate that sodium retention with human growth hormone in man is independent of aldosterone secretion.

Received October 20, 1960.

* Presented in part by Dr. E. G. Biglieri at the First International Congress of Endocrinology, Copenhagen, Denmark, July 18–23, 1960.

† This work was carried out under a grant (A-3870) from the National Institute for Arthritis and Metabolic Diseases, United States Public Health Service.

A grant-in-aid by Merck & Co., Inc. (Dr. Elmer Alpert) is gratefully acknowledged.

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INTRODUCTION

RETENTION of sodium and expansion of the extracellular fluid volume has been observed in man after the administration of human growth hormone (HGH) (1), and in acromegaly (2). Early studies with bovine growth hormone demonstrated an increase in urinary aldosterone (3), which subsequently was not observed with purified growth hormone preparations (4). HGH produced no changes in urinary aldosterone in normal subjects, even though sodium retention occurred (1). However, increases in aldosterone secretion have been observed in 2 patients with panhypopituitarism and in a patient with osteoporosis (5).

This study was undertaken to elucidate the mechanism of acute retention of sodium in man and to assess the role of changes in aldosterone secretion after the intramuscular injection of HGH in normal subjects, a bilaterally adrenalectomized patient, and 2 patients with primary aldosteronism, before and after operation.

MATERIALS AND METHODS

Studies with human growth hormone were made on subjects receiving a constant metabolic diet and constant fluid intake. Control values were determined after a minimum of four days of equilibration. Sodium intake was between 110 and 120 mEq. per twenty-four hours, except in the bilaterally adrenalectomized patient, whose intake was 180 mEq. per twenty-four hours. Four-hour or eight-hour urine collections were analyzed for sodium and potassium by internal standard flame photometry. Urine in 24-hour pools was analyzed for nitrogen by the Kjeldahl technique the day before HGH was given, and again the day after the study. Serum electrolyte levels were determined daily. Serum free fatty acid (FFA) levels (6) were determined at 8 a.m. and 4 p.m. on the control and experimental days.

On the day of the study, a total dose of HGH ranging from 8.2 to 10.0 mg. was given, half at 8 a.m. and half at 12 noon. The endogenous creatinine clearance of eight or twelve hours was determined as an index of the glomerular filtration rate. The patients were weighed daily.

The 24-hour urinary excretion of aldosterone was determined by the double isotope derivative method (7). The human growth hormone used was derived from two sources: an amorphous preparation from the laboratories of Professor C. H. Li (8) and a crystalline preparation from the research laboratories of the Merck Institute (9), made available by Dr. E. Alpert. Growth hormone subfractions were prepared by Professor C. H. Li *et al.* (10), using partial hydrolysis with chymotrypsin of whole bovine or human growth hormone.

Five subjects without cardiac or renal disease or evidence of sodium or fluid retention were hospitalized in the metabolic ward throughout the study.

Two patients (R.T. and L.B.) with classic primary aldosteronism, described in detail elsewhere (11), had potassium deficits in excess of 600 mEq. and were refractory to Pitressin after failure to concentrate urine following an overnight period of dehydration. In each there was virtually no change in sodium balance, potassium balance, body weight, or urinary aldosterone after the daily intramuscular administration of 20 mg. of desoxycorticosterone acetate (DCA) for four days. Doses were calculated to equal approximately the endogenous secretion of aldosterone.

RESULTS

The activity of the HGH was demonstrated in each instance by nitrogen retention and a rise in the concentration of serum FFA. At the almost identical dosage levels used, there was no significant difference between the 2 preparations of HGH in the magnitude of their effect on these parameters.

Miscellaneous subjects (Table 1; Figs. 1 and 2)

Sodium retention, reported as the deviation from the control, ranged from 15 to 53 mEq. per twenty-four hours, with a mean of 36 mEq. Potassium retention ranged from 6 to 60 mEq. per twenty-four hours, with a mean retention of 31 mEq. Among the 5 patients tested with HGH, the mean urinary aldosterone excretions on control and experimental days were 7.7 μg. and 6.4 μg. per twenty-four hours, respectively. Individual variations were slight and in all cases there was a tendency toward a decrease in urinary aldosterone on the experimental day. Return of natriuresis to the control level or beyond occurred in all subjects the day after administration of HGH. Though retention was slight in Subjects S.H. and O.M., the urinary sodium fell to lower levels than in any of the control periods, and there was a small loss after discontinuing HGH. Mean retention of nitrogen was 1.5 Gm. per twenty-four hours on the day of HGH administration. One of the three more pronounced responses is shown in Figure 1.

TABLE 1. Acute responses to human growth hormone

SUBJECT	TREATMENT mg HGH	DEVIATION FROM CONTROL URINARY N gm/d.	DEVIATION FROM CONTROL mEq/24 hrs.		URINARY ALDOSTERONE μg/24 hrs.	
			URINARY Na	URINARY K	CONTROL	EXPERIMENTAL
B.B., 54, ♂	10.0 (MERCK)	1.0	50	31	8.7	4.4
S.H., 31, ♂	10.0 (MERCK)	1.9	17	14	11.0	12.8
R.T.*, 25, ♂	10.0 (MERCK)	0.9	26	60	2.5	2.5
D.V., 54, ♂ a.	10.0 (LI)	1.3	52	46.5	4.7	2.2
b.	8.2 (LI)	2.4	53	31	4.7	3.4
M.S., 52, ♀	10.0 (LI)	2.0	-	-	11.4	10.6
O.M., 60, ♂	8.2 (LI)	1.0	15	6	11.0	9.0
	RANGE	0.9-2.0	15-53	6-60	2.5-11.4	2.2-12.8
	MEAN	1.5	36	31	7.7	6.4

* One year after unilateral adrenalectomy.

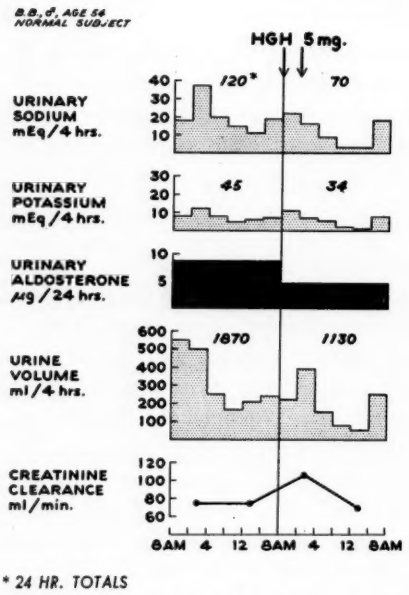

FIG. 1. Effects of administration of human growth hormone in a normal subject.

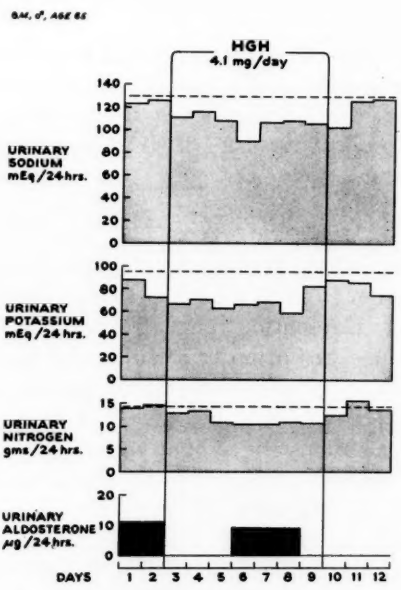
O.M., ♂, AGE 65
HGH
4.1 mg/day
URINARY SODIUM mEq/24 hrs.
URINARY POTASSIUM mEq/24 hrs.
URINARY NITROGEN gms/24 hrs.
URINARY ALDOSTERONE µg/24 hrs.
DAYS
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 2. Effects of prolonged administration of human growth hormone in a control patient.

A more prolonged study in Patient O.M. (Fig. 2) revealed a small but continued retention of sodium without a rise in aldosterone secretion.

Diabetes insipidus (*Fig. 3*)

A patient with diabetes insipidus was studied in a similar manner. He displayed a response comparable in all respects to that in the normal subjects except for a slight and insignificant rise in urinary aldosterone. However, after each injection of HGH there was a transient increase in the osmolarity of the urine above that of the plasma. Although no effort was made to control the fluid intake, his urine output was decreased by 3 liters for that 24-hour period.

Primary aldosteronism (*Figs. 4 and 5*)

In Patients R.T. and L.B., sodium retentions were 79.0 and 87.0 µg. per twenty-four hours and weight gains 0.5 and 0.7 Kg., respectively. Urinary aldosterone excretions fell from 50 to 18 µg. and from 37 to 15 µg. per twenty-four hours, respectively. Potassium retentions were 27 and 65 mEq. per twenty-four hours, respectively. Nitrogen retention was 1.2 Gm. per twenty-four hours in each subject. There was no significant change in

creatinine clearance or serum electrolyte levels. In Patient L.B., the maximal decrease in urinary aldosterone occurred during the period of greatest sodium retention. The response in Patient R.T. was not as prompt and the fall in urinary aldosterone on the day of administration of HGH was not associated with maximal retention of sodium.

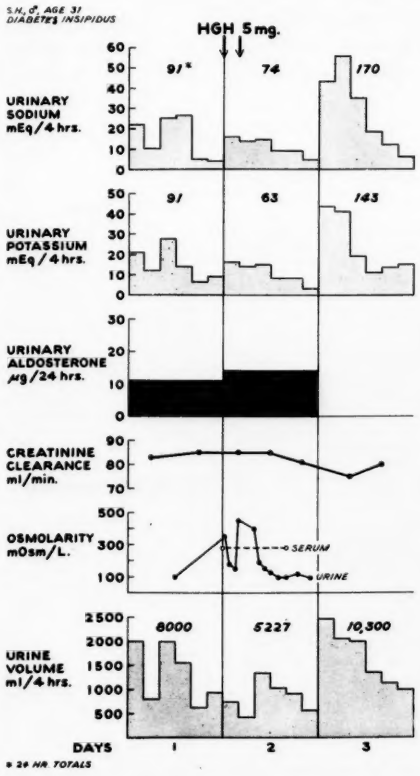

FIG. 3. Effects of administration of human growth hormone in a patient with diabetes insipidus.

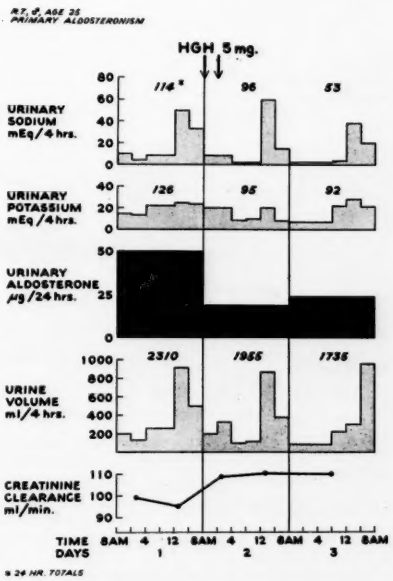

FIG. 4. Effects of administration of human growth hormone in Patient R.T. (primary aldosteronism).

Patient R.T. was studied after removal of an adrenal adenoma (Fig. 6), under the same balance conditions as before operation. On this occasion, sodium retention was 26 mEq. and potassium retention 60 mEq. per twenty-four hours. Urinary aldosterone showed no change from the control value, 2.5 μg. per twenty-four hours. Potassium repletion had probably occurred at this time, as the serum potassium level, the renal concentrating ability and the exchangeable sodium had returned to normal.

Bilateral adrenalectomy (Fig. 7)

A patient who had undergone bilateral adrenalectomy for Cushing's disease was studied while receiving dexamethasone,[1] a constant high-salt intake, but no mineralocorticoids. His sodium retention was 25 mEq. per twenty-four hours after intramuscular administration of 10 mg. of HGH;

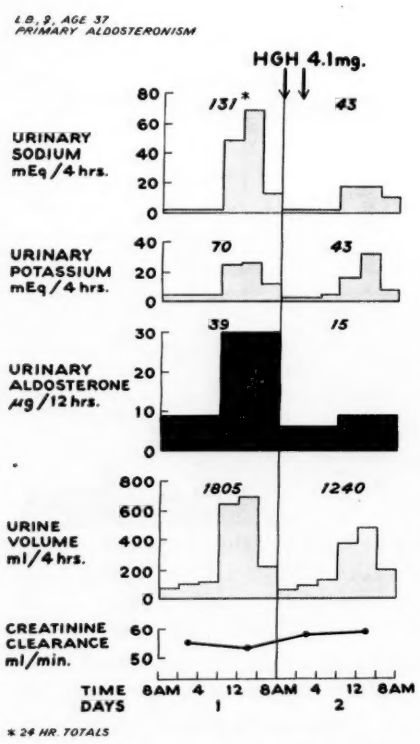

FIG. 5. Effects of administration of human growth hormone in Patient L.B. (primary aldosteronism).

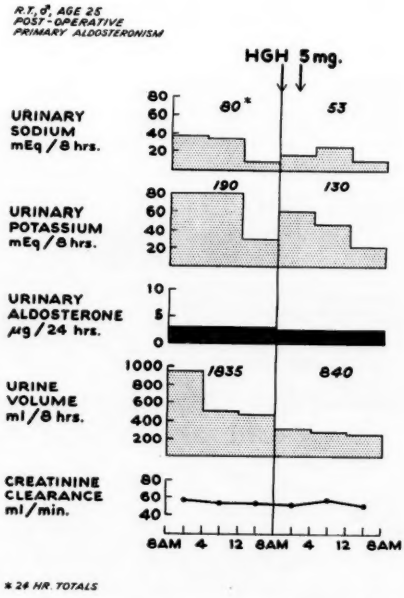

FIG. 6. Effects of administration of human growth hormone in Patient R.T. (see Fig. 4), after operation.

this level was reproduced on two occasions. When the test was repeated while he was receiving a maintenance dose of desoxycorticosterone acetate (DCA) (1 mg. per twelve hours), the sodium retained was 17 mEq. per twenty-four hours. The study was performed a third time, after "escape" from the effects of 10 mg. of DCA intramuscularly per day. A new sodium balance had been achieved, and sodium retention reached 32 mEq. per

[1] Kindly supplied by Dr. Elmer Alpert of Merck & Co., Inc., Philadelphia, Pennsylvania.

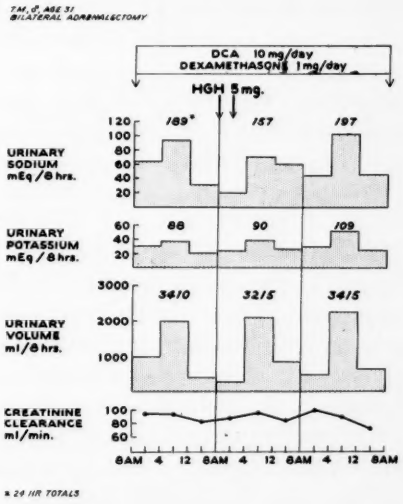

FIG. 7. Effects of administration of human growth hormone in an adrenalectomized patient after "escape" from DCA.

twenty-four hours. No significant change had occurred in endogenous creatinine clearance or serum electrolyte levels.

Growth hormone subfraction (Table 2)

Administration of 10 mg. of alpha core human growth hormone, a chymotrypsin digest of HGH, produced sodium, potassium and nitrogen retention and a rise in the concentration of fasting serum FFA comparable to

TABLE 2. Acute responses to growth hormone subfractions

SUBJECT	TREATMENT	DEVIATION FROM CONTROL mEq/24 hrs.		URINARY ALDOSTERONE μg/24 hrs.	
		URINARY Na	URINARY K	CONTROL	EXPERIMENTAL
D.V., 54, ♂	ALPHA CORE HUMAN GROWTH HORMONE 10 mg. I.M.	+53.0	+37.0	4.7	3.4
	ALPHA CORE BOVINE GROWTH HORMONE 50 mg. I.M.				
	a.	+39.0*		4.7	10.0*
	b.	+ 3.0			4.0
	c.	+ 2.0			
O.M., 60, ♂	ALPHA CORE BOVINE GROWTH HORMONE 20 mg. I.M.	−15.0	− 5.0	12.0	6.0

* EXQUISITE PAIN AT INJECTION SITE FOR 12 HOURS

levels produced by whole HGH of the same dosage. Alpha core bovine growth hormone in doses of 20 and 50 mg. produced no sodium or nitrogen retention or any change in urinary aldosterone, in 3 of 4 studies. In the remaining study the patient experienced severe pain at the site of injection; sodium retention was 39.0 mEq., and there was a rise in aldosterone excretion from 4.7 to 10.0 μg. per twenty-four hours.

DISCUSSION

The mean acute sodium retention observed in the normal group following intramuscular administration of HGH was comparable to that previously reported (1, 5). The potassium retention was in excess of that attributable to the nitrogen retention, and was an effect opposite to that found with aldosterone. Sodium retention occurred without significant changes in the endogenous creatinine clearance.

The patient with diabetes insipidus was of particular interest, since he was able to concentrate urine to an osmolarity above serum osmolarity in the specimens voided immediately after the administration of HGH. This suggests the presence of some antidiuretic activity in the preparation of HGH used, although a routine biologic assay for antidiuretic hormone, carried out by the manufacturer, yielded negative results.

The most striking sodium retention occurred in the 2 patients with primary aldosteronism in whom expanded extracellular fluid volumes had previously been demonstrated. Further attempts to increase sodium retention with DCA, aldosterone and 2 and 3 liters of isotonic saline over a one-hour period had been unsuccessful. However, the observed retention of sodium and water after administration of HGH indicated that further expansion had occurred by a mechanism not dependent upon aldosterone, or upon a decrease in endogenous creatinine clearance. The response was much more marked in Patient L.B. than in Patient R.T. The prompt decreases in urinary aldosterone probably represented responses to an increase in extracellular fluid (12), as the fall in aldosterone, especially in L.B., occurred during sodium retention and weight gain. The prompt reduction in urinary aldosterone in Patient R.T. on the day of HGH administration, accompanied by a sodium retention of only 18 mEq. per twenty-four hours, cannot be readily explained. The postoperative study on Patient R.T. revealed an essentially normal sodium response to HGH when potassium deficits had been replenished. Potassium retention was excessive.

The bilaterally adrenalectomized patient exhibited sodium and potassium retention similar to that in the normal subjects, whether in the absence of salt-retaining hormones or when taking 1 mg. of DCA per day. When "escape" had been achieved after large doses of DCA, with a cumulative potassium deficit of less than 50 mEq., the response was slightly greater, but was not of the magnitude seen in the patients with primary

aldosteronism. Similar responses have been observed in adrenalectomized rats receiving bovine growth hormone, with or without adrenocortical extract (13).

HGH apparently does not act through changes in aldosterone secretion, since 1) it could not be operative by this route in the adrenalectomized patient, and 2) a fall in urinary aldosterone occurred concurrently with an exaggerated retention of sodium in the patients with primary aldosteronism. The fact that HGH produced essentially normal responses in sodium retention in either the absence or the presence of excessive amounts of salt-retaining hormones suggests that the latter are not "permissively" essential for this effect.

In primary aldosteronism, the marked retention of sodium produced by HGH during a one-day or two-day period may be a function of the marked potassium depletion. This could represent an exaggeration of the normal response, for normal subjects undergoing potassium depletion exhibit sodium retention and, subsequently, natriuresis upon potassium repletion (14, 15). At this point, it is difficult to localize the site of action of sodium retention. The absence of serum electrolyte changes, the slight increases in creatinine clearance, and the retention of both sodium and potassium with gain in weight, suggest that the locus of action of HGH may well be at the tubular level. Renal effects of growth hormone have been noted previously. A renotropic effect has been ascribed to bovine growth hormone administered to dogs, because increases in inulin and PAH clearances were seen after five days of treatment, but not immediately (16). Lack of growth hormone may well play an important role in the diminished renal function of hypophysectomized subjects (17).

Li (18) has suggested that the various growth hormones have similar active cores. With the use of smaller growth hormone molecules prepared by chymotrypsin digestion, dissociation of function might be anticipated. However, alpha core HGH produced sodium, nitrogen and FFA changes similar to those seen with the whole molecule. The preparations of bovine alpha core growth hormone employed in this study proved to be as inactive as the whole bovine growth hormone. However, an interesting observation was made at the time of the third injection of similar bovine alpha core in Patient D.V. that may account for the sodium retention found after administration of an earlier preparation (19). Exquisite and prolonged pain at the site of injection produced not only an increase in urinary aldosterone but an increase in sodium retention not unlike the response to stress previously observed (20).

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THE DIAGNOSTIC VALUE OF SODIUM TOLBUTAMIDE IN HYPOGLYCEMIC STATES*†

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ABSTRACT

Intravenous sodium tolbutamide tests were performed in patients with 1) proven insulomas, 2) functional hyperinsulinism, 3) mild diabetes mellitus and associated postprandial hypoglycemia, 4) severe adrenal insufficiency, and 5) liver disease. Healthy subjects were used as controls. Administration of sodium tolbutamide to patients with functioning islet-cell adenomas usually produced a greater degree of hypoglycemia than was observed in the other subjects studied. Of much greater significance, however, were the prolonged periods of hypoglycemia in the insuloma patients. After removal of the insulomas the response to tolbutamide became normal. Persistence of tolbutamide-induced hypoglycemia: 1) is a valuable adjunct in the diagnosis of insuloma, 2) is due, in part, to release of excessive amounts of insulin from the insuloma, and 3) is supportive evidence that the primary mechanism of action of the sulfonylureas is release of pancreatic insulin. From a clinical point of view the intravenous tolbutamide test is of greatest value in the differentiation between organic and functional hyperinsulinism.

INTRAVENOUS administration of sodium tolbutamide to normal subjects results in a rapid fall of the blood sugar level for thirty to forty-five minutes followed by a secondary rise to within the normal range between one and a half and three hours after administration of the drug (1, 2). The initial hypoglycemia is believed to be due to rapid release of insulin from the pancreatic beta cells (3–5). The secondary rise is due in part to activation of counter-regulatory mechanisms such as glycogenolysis induced by release of epinephrine. The present study was initiated to explore

Received October 5, 1960.

* Presented in part before the Thirty-Second Annual Meeting of the Central Society for Clinical Research, Chicago, November 6–7, 1959 and at the Forty-Second Meeting of The Endocrine Society, Miami Beach, Florida, June 9–11, 1960.

† This work was supported in part by Research Grants A-888 and A-2244 from The National Institute for Arthritis and Metabolic Diseases, U.S.P.H.S. and by a grant from the Upjohn Company, Kalamazoo, Michigan.

the effect of intravenous sodium tolbutamide on the blood sugar levels of patients with functioning pancreatic islet-cell adenomas. Patients with other forms of spontaneous hypoglycemia were studied similarly. The results indicate 1) that sulfonylurea drugs induce the release of large amounts of insulin from pancreatic insulomas and thus cause prolonged hypoglycemia; 2) that this phenomenon may serve as a valuable adjunct in the diagnosis of insuloma; and 3) that release of insulin from insulin-producing cells is a primary action of sulfonylurea compounds.[1]

SUBJECTS AND METHODS

Intravenous sodium tolbutamide tests were performed in the following groups of subjects: *a*) 20 healthy persons; *b*) 6 patients with proven insulomas; *c*) 11 patients with functional hyperinsulinism; *d*) 6 patients with postprandial hypoglycemia associated with early diabetes mellitus; *e*) 11 patients with severe adrenal insufficiency (4 after total adrenalectomy and 7 in association with Addison's disease); *f*) 17 patients with liver disease (6 with portal cirrhosis, 5 with post-necrotic cirrhosis and 6 with acute hepatitis).

All subjects were given a high carbohydrate diet for at least three days before each study. One gram of sodium tolbutamide[2] diluted with 10 ml. of distilled water was administered intravenously over two minutes to all but 3 subjects. Two healthy control subjects and 1 patient with an insuloma received 30 mg. of the drug per Kg. of body weight.

Blood samples were obtained at intervals of fifteen to thirty minutes for three hours, and blood sugar concentration was determined by the Somogyi-Nelson method (8). For the glucagon tests, 1 mg. of crystalline glucagon was administered by intravenous injection over a period of four minutes.[3]

The patients with adrenal insufficiency received no more oral glucocorticoids and were maintained with desoxycorticosterone in oil intramuscularly for three to eleven days (mean, seven days) before performance of the tolbutamide test.

In the patients with liver disease the severity of hepatic damage was assessed by clinical findings (such as ascites and esophageal varices), the results of liver function tests (bilirubin, bromsulfalein retention, serum protein levels, prothrombin time, cephalin flocculation, and serum alkaline phosphatase and transaminase activities) and the pathologic findings in liver biopsy specimens. On the basis of these criteria the severity of liver disease was classified as follows: *a*) mild (2 patients with portal cirrhosis and 4 patients with acute hepatitis); *b*) moderately severe (1 patient with portal cirrhosis, 1 with post-necrotic cirrhosis, and 2 with hepatitis); or *c*) severe (3 patients with portal cirrhosis and 4 with post-necrotic cirrhosis).

Intravenous insulin tolerance tests were performed in a healthy 32-year-old man, before and after a state of fasting hypoglycemia had been induced. Chlorpropamide was used to produce sustained lowering of the fasting blood sugar level. With a dosage of

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[1] Preliminary reports of these studies have appeared in abstract form (6, 7).

[2] Kindly supplied by Dr. C. J. O'Donovan, The Upjohn Company, Kalamazoo, Michigan.

[3] Kindly supplied by Dr. W. R. Kirtley, Eli Lilly Research Laboratories, Indianapolis, Indiana.

0.875 Gm. orally per day the fasting blood sugar levels ranged between 50 and 60 mg. per 100 ml. in this subject. Glucagon-free insulin[3] was used for the intravenous insulin tolerance tests.

RESULTS

The results obtained with the tolbutamide test in 2 patients with proven insuloma are shown in Figures 1 and 2. Figure 1 demonstrates a fall in the blood sugar level to 17 mg. per 100 ml. following administration of tolbutamide to Patient M.B. Severe hypoglycemia persisted for the period of three hours. Figure 1 also indicates that there was no appreciable change in the blood sugar level following a control injection of physiologic saline. Two years following a subtotal pancreatectomy which included the insuloma, the tolbutamide test yielded a normal result, though the glucose tolerance test indicated the presence of mild pancreatic diabetes.

Figure 2 illustrates the salient findings in a man with diabetes mellitus

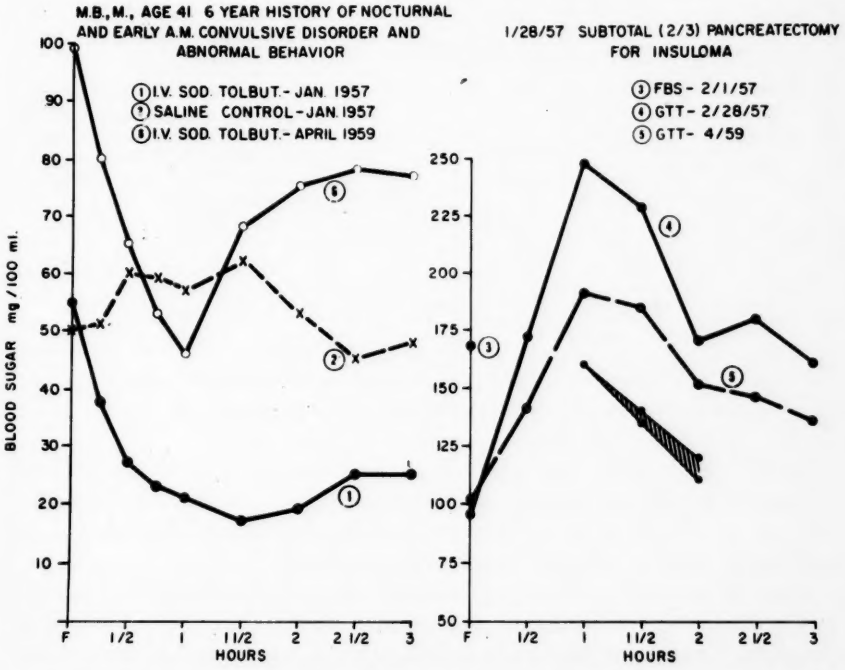
M.B., M., AGE 41 6 YEAR HISTORY OF NOCTURNAL AND EARLY A.M. CONVULSIVE DISORDER AND ABNORMAL BEHAVIOR
1/28/57 SUBTOTAL (2/3) PANCREATECTOMY FOR INSULOMA
① I.V. SOD. TOLBUT.- JAN. 1957
② SALINE CONTROL - JAN. 1957
⑤ I.V. SOD. TOLBUT.- APRIL 1959
③ FBS - 2/1/57
④ GTT - 2/28/57
⑤ GTT - 4/59
BLOOD SUGAR mg/100 ml.
HOURS
F 1/2 1 1 1/2 2 2 1/2 3

FIG. 1. Intravenous tolbutamide tests in a patient with a functioning pancreatic islet-cell adenoma (insuloma). Postoperative glucose tolerance tests are shown on the right side of the figure. The shaded area indicates criteria employed for the interpretation of the oral glucose tolerance test. A diabetic curve is one in which all points lie at or above the upper border of the shaded triangle.

and an insuloma. This patient (N.J.) was previously reported by Dr. R. D. Gittler (9) who was kind enough to perform the tolbutamide test (10). After intravenous administration of sodium tolbutamide there was a fall in the blood sugar level from 72 mg. to 26 mg. per 100 ml., with persistence of hypoglycemia for three hours. In contrast to the findings in Patient M.B., there was a fall of the blood sugar level during the control test also, but at a less rapid rate and not to the same low level. A 72-gram pancreatic

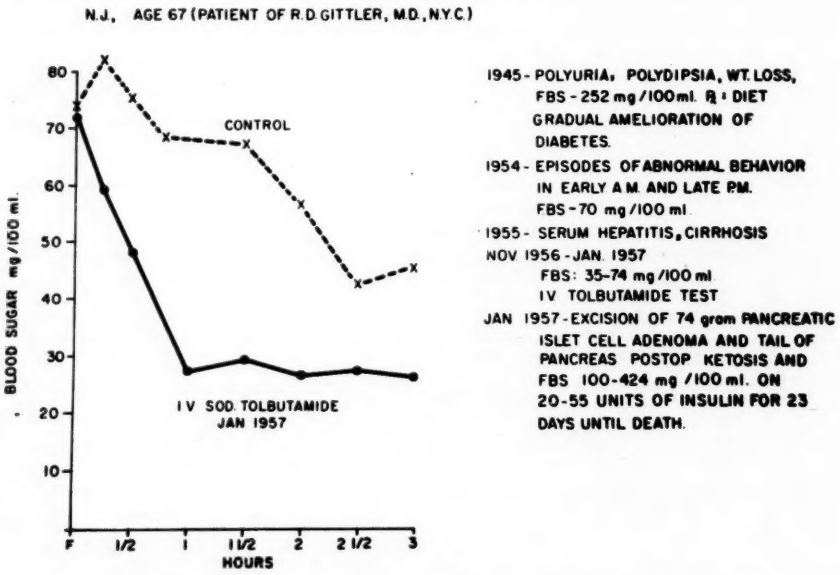
N.J., AGE 67 (PATIENT OF R.D.GITTLER, M.D., N.Y.C.)
BLOOD SUGAR mg/100 ml.
80
70
60
50
40
30
20
10
CONTROL
I V SOD. TOLBUTAMIDE
JAN 1957
F
1/2
1
1 1/2
2
2 1/2
3
HOURS
1945 - POLYURIA, POLYDIPSIA, WT. LOSS, FBS - 252 mg/100 ml. R : DIET GRADUAL AMELIORATION OF DIABETES.
1954 - EPISODES OF ABNORMAL BEHAVIOR IN EARLY A.M. AND LATE P.M. FBS - 70 mg/100 ml.
1955 - SERUM HEPATITIS, CIRRHOSIS
NOV 1956 - JAN. 1957
FBS: 35-74 mg/100 ml.
I V TOLBUTAMIDE TEST
JAN 1957 - EXCISION OF 74 grm PANCREATIC ISLET CELL ADENOMA AND TAIL OF PANCREAS POSTOP KETOSIS AND FBS 100-424 mg/100 ml. ON 20-55 UNITS OF INSULIN FOR 23 DAYS UNTIL DEATH.

FIG. 2. Intravenous tolbutamide test in a patient with diabetes mellitus and a functioning pancreatic islet-cell adenoma (insuloma).

adenoma was excised from the tail of the pancreas. Postoperatively the patient suffered from ketosis, and fasting blood sugar levels ranged between 100 mg. and 424 mg. per 100 ml. while he was receiving 20 to 25 units of insulin for the last twenty-three days of his life (9).

Figure 3 shows the results of 8 intravenous tolbutamide tests performed in 6 patients with insulomas, contrasted with the findings in 20 healthy subjects. The results are expressed as absolute blood sugar levels in mg. per 100 ml., and also as the percentage decrease from fasting blood sugar values. Tolbutamide caused greater hypoglycemia in the insuloma patients but it was the persistence of hypoglycemia which distinguished clearly the insuloma group from the normal group. Figure 3 shows the upper and lower ranges of blood sugar values for the two groups, in addition to the mean

values, standard errors and P values. There is no overlap in values between one and a half and three hours, even when considered on the basis of percentage decreases from fasting levels. In 1 of the insuloma patients the test had to be terminated one and a half hours after the injection of tolbutamide because of loss of consciousness due to the severity and persistence of hypoglycemia.

Tolbutamide tests were performed in 5 of the insuloma patients after

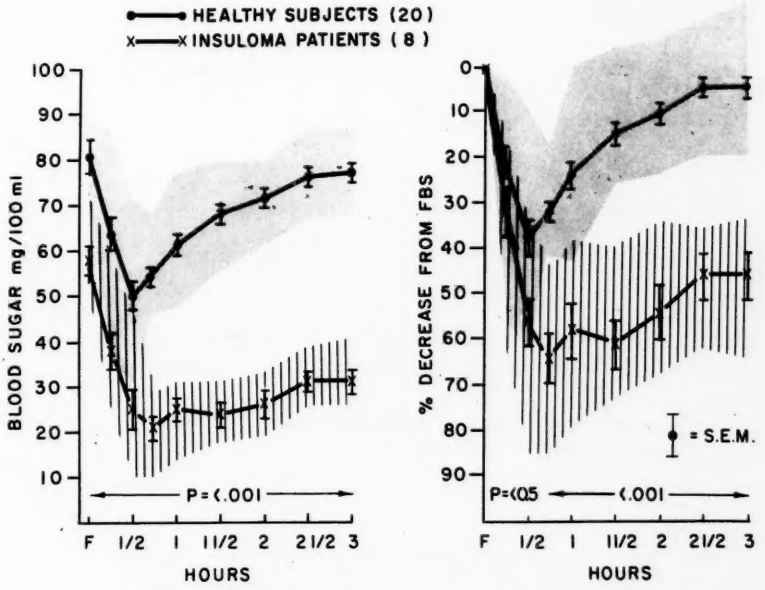

FIG. 3. Intravenous tolbutamide tests in 20 healthy subjects and in 6 insuloma patients. The shaded zones represent the ranges of blood sugar values for the two groups.

resection of the pancreatic islet-cell tumors. The results fell within the normal range.

The response to tolbutamide in 11 patients with functional hyperinsulinism (Fig. 4) was very similar to that in healthy subjects, and clearly separated these patients from those with insulomas.

The tolbutamide response in patients with mild diabetes mellitus associated with secondary hypoglycemia (11) is shown in Figure 5. In this group the blood-sugar-lowering action of tolbutamide was delayed and was less marked than in normal subjects, and the secondary rise was in the normal range.

The persistence of the tolbutamide-induced hypoglycemia for three

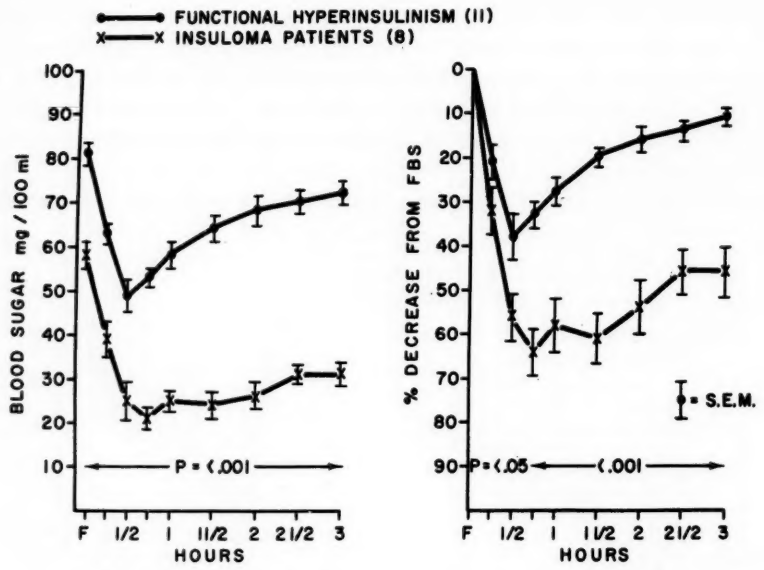

FIG. 4. Intravenous tolbutamide tests in patients with functional hyperinsulinism and in patients with insuloma.

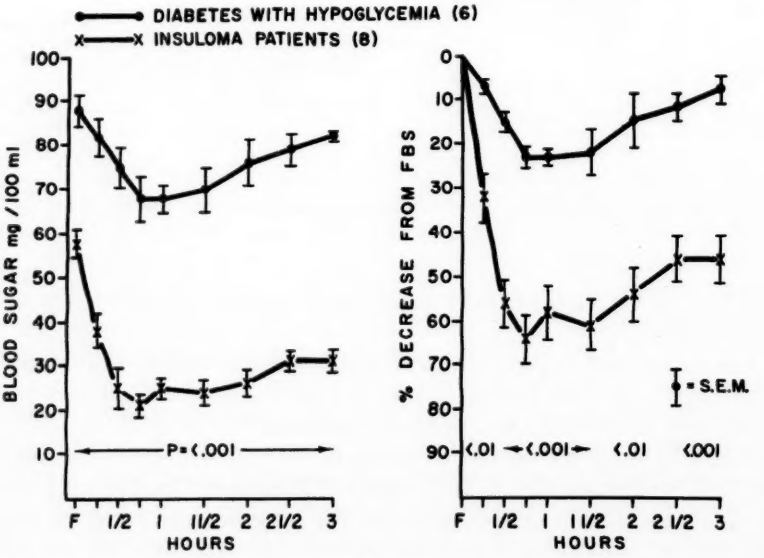

FIG. 5. Intravenous tolbutamide tests in patients with diabetes mellitus and secondary hypoglycemia and in patients with insuloma.

hours in the insuloma patients may have been due to: 1) the release of large amounts of insulin from the adenomas; 2) hypoglycemia-unresponsiveness as a result of decreased glycogenolysis or gluconeogenesis; or 3) a combination of these factors.

To test the adequacy of hepatic glycogen stores, intravenous glucagon tests were performed in 3 insuloma patients. In all 3, the hyperglycemic responses were within normal limits. The maximal increases in blood sugar concentration after injection of glucagon were 55, 41 and 31 mg. per 100 ml. (normal range, 30 to 70 mg. per 100 ml.).

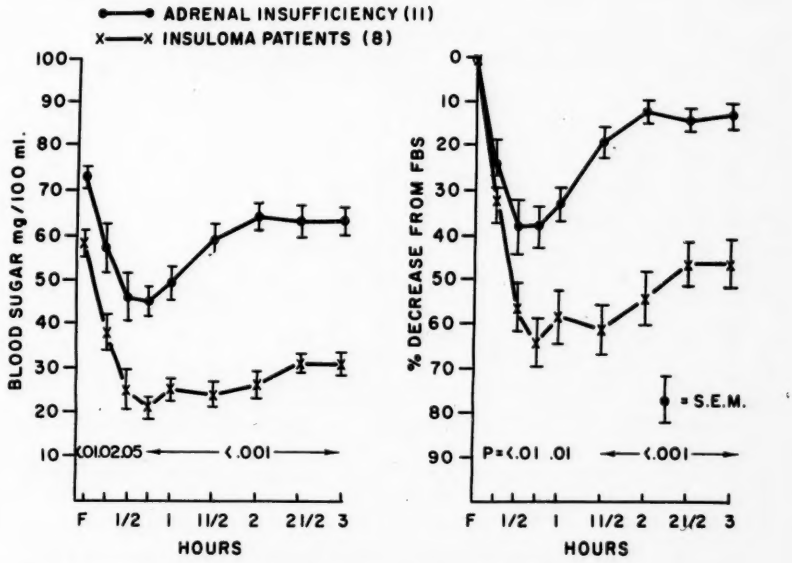

FIG. 6. Intravenous tolbutamide tests in patients with adrenal insufficiency and in patients with insuloma.

Tolbutamide tests were performed on 11 patients with severe adrenal insufficiency who were maintained with desoxycorticosterone and were not receiving glucocorticoids (Fig. 6). Again the most significant differences between this group and the insuloma group were observed between one and a half and three hours after the injection. Patients with adrenal insufficiency showed a good, but slightly subnormal, recovery from hypoglycemia.

The results obtained with the tolbutamide test in 17 patients with liver disease are illustrated in Figure 7. The lowest fasting blood sugar level was 62 mg. per 100 ml. The hypoglycemic response to tolbutamide was less pronounced and slightly delayed in these patients as compared with either

the insuloma patients or with the healthy subjects. Between one and a half and three hours after the injection, the blood sugar concentration in the patients with liver disease was distinctly less depressed from the fasting level than in the insuloma group. Glucagon tests performed in several of these patients showed subnormal responses (increases in the blood sugar level of less than 25 mg. per 100 ml.).

When a comparison is made of the lowest blood sugar levels reached after injection of tolbutamide in all patients of the 6 groups, it becomes apparent that although the lowest levels were reached in the insuloma patients,

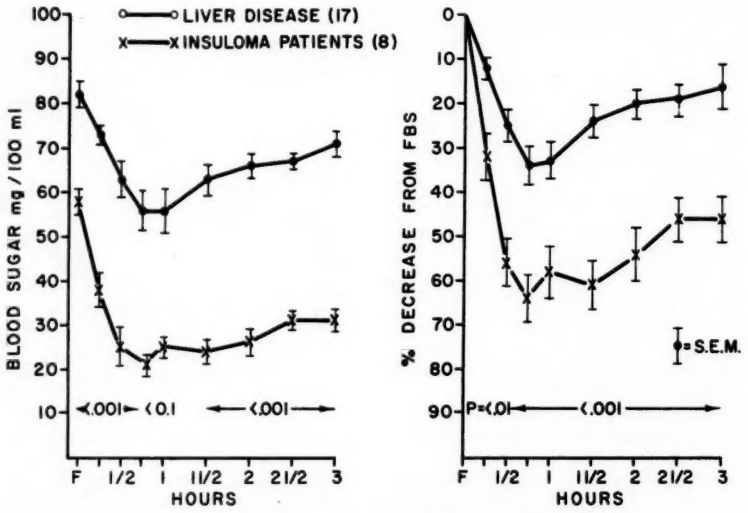

FIG. 7. Intravenous tolbutamide tests in patients with liver disease and in patients with insuloma.

there was considerable overlapping among the groups, particularly when the values were expressed as the percentage decrease from fasting levels (Fig. 8).

In Figure 9 a comparison is made of the highest blood sugar levels reached at one and a half to three hours after the injection of the drug for all patients in the 6 groups. The insuloma patients are clearly demarcated from the normal subjects, from the two types of patient with functional hypoglycemia, and from the patients with adrenal insufficiency. Figure 9 shows the longer duration of hypoglycemia in the insuloma patients as compared to the others. There was some overlapping between the insuloma and the liver disease groups, although the means of the groups were significantly different.

In a healthy subject, the response to the tolbutamide test could be duplicated by the response to administration of 0.05 unit of insulin per Kg. of body weight (Fig. 10). The blood sugar response to tolbutamide in insuloma patients could not be reproduced in this healthy subject, even with 0.6 unit of insulin per Kg. given in fractional doses. It could be reproduced with 0.6 unit of insulin per Kg., if administered when the patient was already in a state of chlorpropamide-induced fasting hypoglycemia. The same healthy subject was given a diet containing 50 Gm. of carbohydrate and maintenance calories for seven days. Intravenous administration of

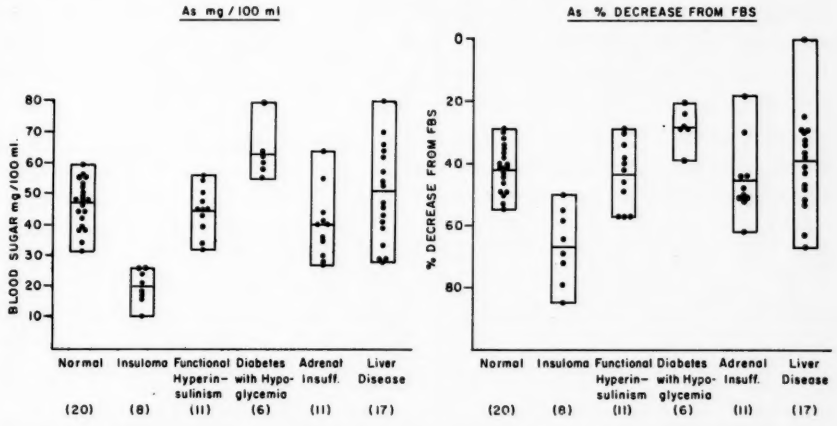

FIG. 8. Lowest blood sugar levels after intravenous administration of sodium tolbutamide in normal subjects, patients with insuloma, and patients with other forms of spontaneous hypoglycemia.

0.44 unit of insulin per Kg. in a single dose did not reproduce the tolbutamide-induced blood sugar response seen in insuloma patients (Fig. 10).

The value of the tolbutamide test in excluding the diagnosis of insuloma is illustrated by the histories of the following 2 patients in whom insulomas were strongly suspected. One was a 66-year-old male admitted to the Emergency Room of University Hospital in a comatose state, with a blood sugar level of 13 mg. per 100 ml. He regained consciousness promptly after an infusion of 5 per cent glucose in water was begun. He admitted to having partaken of a poor diet and consuming generous amounts of alcohol. Liver function and urinary excretion of 17-hydroxycorticoids and 17-ketosteroids were normal. The other patient was a 21-year-old female with a history of nocturnal and daytime convulsions. Her fasting blood sugar level was 62 mg. per 100 ml. and during the course of a glucose tolerance test the level fell to 29 mg. per 100 ml. at four hours. The diagnosis of insuloma was

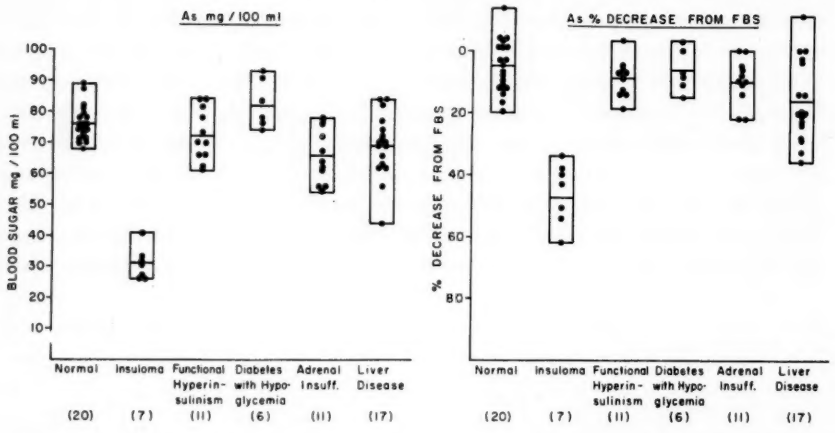

FIG. 9. Highest blood sugar level at 1½–3 hours after intravenous administration of sodium tolbutamide in normal subjects, patients with insuloma, and patients with other forms of spontaneous hypoglycemia.

ruled out in both patients by a number of criteria, including fasting blood sugar levels within the range seen in normal subjects during a three-day period of feeding a provocative diet containing 50 Gm. of carbohydrate, 50 Gm. of protein and 1,200 calories followed by complete starvation for sixty-three hours coupled with vigorous exercise during the last two hours.

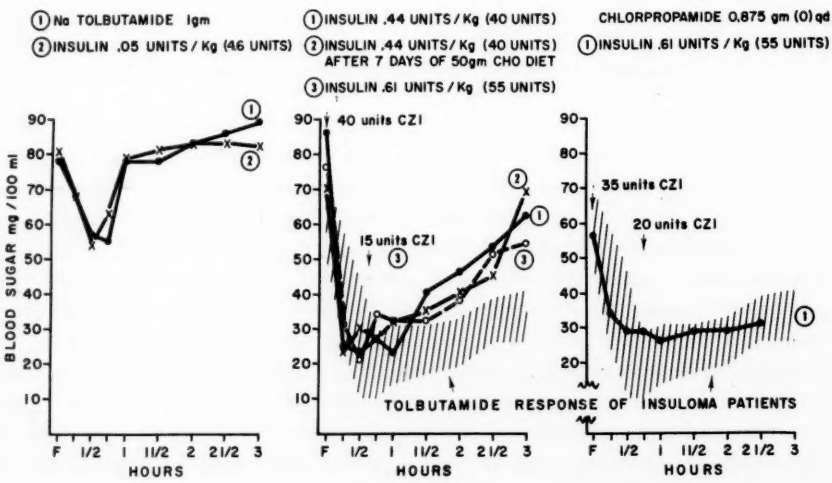
① No TOLBUTAMIDE 1gm
② INSULIN .05 UNITS / Kg (4.6 UNITS)
① INSULIN .44 UNITS / Kg (40 UNITS)
② INSULIN .44 UNITS / Kg (40 UNITS) AFTER 7 DAYS OF 50gm CHO DIET
③ INSULIN .61 UNITS / Kg (55 UNITS)
CHLORPROPAMIDE 0.875 gm (0) qd
① INSULIN .61 UNITS / Kg (55 UNITS)
BLOOD SUGAR mg / 100 ml
40 units CZI
15 units CZI
35 units CZI
20 units CZI
TOLBUTAMIDE RESPONSE OF INSULOMA PATIENTS
F 1/2 1 1 1/2 2 2 1/2 3
HOURS

FIG. 10. Effect of intravenous insulin and intravenous sodium tolbutamide in a healthy man. Comparison with response to intravenous tolbutamide in insuloma patients.

Tolbutamide tests were performed in both patients. The lowest blood sugar level reached after tolbutamide was 43 mg. per 100 ml. At three hours after tolbutamide, the blood sugar levels were 81 and 63 mg. per 100 ml.—depressions of 0 and 9 per cent, respectively. Thus, it would appear that this test can spare the insuloma "suspect" much time, money and inconvenience.

DISCUSSION

Intravenous administration of sodium tolbutamide to 6 patients with functioning islet-cell adenomas produced profound decreases in the concentration of blood sugar. The most significant aspect of this response was the persistence of hypoglycemia for the entire period of the three-hour test. The same response to tolbutamide has been obtained in 10 patients with insuloma by Pfeiffer *et al.* (5), Frawley *et al.* (12), Floyd (13), Schwartz (14), Marrack (15) and Beman (16), who tested their patients in a similar manner. In addition, Salassa (17) (1 patient) and Wajchenberg (18) (2 patients) noted severe and persistent hypoglycemia in patients with insulomas who were given a single oral dose of 3 Gm. of tolbutamide. In contrast, in normal subjects, a single oral dose of tolbutamide results in only transient hypoglycemia (1). The nature of the blood sugar response in cases of insuloma separate these patients from: 1) normal subjects; 2) patients with functional hyperinsulinism; 3) patients with mild diabetes mellitus associated with hypoglycemia; 4) patients with severe adrenal insufficiency not treated with glucocorticoids; and 5) patients with liver disease.

These findings indicate that sulfonylurea drugs cause release of large amounts of insulin from the islet-cell adenomas. Supporting evidence for this conclusion comes from the demonstration of a large tolbutamide-induced increase of plasma insulin-like activity in a patient with an insuloma. Pfeiffer and co-workers reported an insuloma patient in whom serum insulin-like activity increased from 2,000 to 6,000 microunits per 100 ml. thirty minutes after administration of tolbutamide (5). These levels are considerably higher than those found in normal subjects after administration of tolbutamide. It is most unlikely that tolbutamide causes release of excessive amounts of insulin from the nontumorous pancreatic tissue of insuloma patients. Hyperinsulinism caused by an insuloma or by administration of excessive amounts of exogenous insulin depresses normal islet-cell function (19). Resection of the insulomas corrected the abnormal response to tolbutamide in our series. In the patient with co-existing diabetes mellitus and organic hyperinsulinism, resection of the insuloma precipitated uncontrolled diabetes and ketosis (9).

Indirect evidence for excessive release of insulin following administration of tolbutamide to insuloma patients can be derived from the studies con-

ducted in the healthy man. The tolbutamide-induced blood sugar response in this subject was duplicated following injection of 0.05 unit of insulin per Kg. of body weight. Four to ten times as much insulin was required in this subject to attain a response approximating the tolbutamide-induced hypoglycemic response in insuloma patients.

It is generally believed that the most important mechanism by which sulfonylurea compounds lower the concentration of glucose in the blood of normal subjects and of diabetic patients is by increased release of endogenous insulin. Although there is a growing body of direct and indirect evidence to support this point of view (3–5), final proof for this hypothesis has not been presented. The data in this report which indicate that tolbutamide causes increased release of insulin from pancreatic islet-cell tumors, constitute further strong supporting evidence for this mechanism of action of sulfonylureas. The data would also indicate that tumorous and nontumorous islet-cell tissues respond to sulfonylurea drugs in the same qualitative way.

The decreased rate of recovery from tolbutamide-induced hypoglycemia in the insuloma patients could, in part, be due to a decrease in the compensatory hepatic release of glucose. This could result from decreased glycogen stores secondary to prolonged hypoglycemia. However, the hyperglycemic response to glucagon in 3 patients with insulomas was in the normal range. A hyperglycemic response to glucagon within the normal range has been observed in 6 other patients with insulomas (20–22). On the other hand, patients with liver disease (in whom hepatic glycogen stores are believed to be decreased) showed a more normal recovery from tolbutamide-induced hypoglycemia. In several of these patients, glucagon tests demonstrated definitely decreased hyperglycemic responses, as has been reported to occur in patients with liver disease (23, 24). It would appear, therefore, that decreased hepatic glycogenolysis secondary to a reduction in hepatic glycogen stores is not an adequate explanation for the prolonged tolbutamide-induced hypoglycemia exhibited by insuloma patients.

Nevertheless, a decrease in hepatic glucose output may contribute significantly to the persistence of tolbutamide-induced hypoglycemia in insuloma patients. Insulin released into the portal circulation has a direct effect in decreasing the hepatic release of glucose (25). Although the effects of insulin upon hepatic metabolism are in some respects still controversial, it appears that increased hepatic production of glucose in response to hypoglycemia is restrained during times of excessive release of insulin (26). Thus, a decreased hepatic output of glucose probably occurs in insuloma patients as a consequence of an excess of insulin in the absence of exhaustion of hepatic glycogen stores.

Recovery from tolbutamide-induced hypoglycemia was much greater in

the patients with adrenal insufficiency than in the insuloma patients. Therefore it is unlikely that decreased gluconeogenesis from protein accounted for the persistent tolbutamide-induced hypoglycemia in the insuloma patients.

From a clinical point of view, the tolbutamide test promises to serve not only as an aid in the differential diagnosis of the various forms of spontaneous hypoglycemia but also as an adjunct in establishing the diagnosis of insuloma. Of the groups of patients tested, those with insulomas exhibited the greatest *maximal depression* of blood sugar concentration after administration of sodium tolbutamide. The degree of depression of the blood sugar level, however, does not have diagnostic value, as overlapping occurred in the various groups tested when this index was used. Of much greater significance was the *persistence* of tolbutamide-induced hypoglycemia for three hours in the insuloma patients; the highest blood sugar level observed three hours after administration of tolbutamide was 41 mg. per 100 ml. When expressed as the percentage fall from fasting levels, this decrease was more than 36 per cent in the insuloma patients. On the other hand, in functional hyperinsulinism, diabetes with hypoglycemia, adrenal insufficiency, and in normal subjects, the greatest decrease three hours after administration of tolbutamide was less than 20 per cent. Only in one of the patients with liver disease was there hypoglycemia-unresponsiveness which approximated that seen in insuloma patients. It may be anticipated that in patients with more severe liver disease, particularly those with severe fasting hypoglycemia, the responses may be indistinguishable from those in insuloma patients. However, the recognition of severe liver disease should present no difficulty in such cases. It is apparent that the tolbutamide test does not replace sound clinical judgment and other laboratory procedures in the differential diagnosis of spontaneous hypoglycemia. It should be regarded as another helpful diagnostic procedure.

Results obtained with the tolbutamide test in leucine-sensitive patients with idiopathic familial hypoglycemia of infancy and childhood (27) are of great interest. Although rare, insulomas do occur in infants and children (22, 28), and the differential diagnosis between idiopathic familial hypoglycemia and hypoglycemia due to insuloma is a difficult one in this age group. Grumbach and Kaplan (29) recently demonstrated an increase in plasma levels of insulin after administration of leucine in patients with familial hypoglycemia. However, leucine sensitivity, when present, does not differentiate idiopathic hypoglycemia from insuloma, since this phenomenon has been described in patients with functioning pancreatic adenoma as well (30). In a leucine-sensitive infant with idiopathic hypoglycemia, Grumbach and Kaplan found a normal response to tolbutamide (29). We have had a similar case. Such results are not surprising, since

leucine appears to induce release of insulin by a mechanism different from that of tolbutamide (31). If further experience should indicate that patients with idiopathic hypoglycemia have consistently normal responses to tolbutamide, the distinction between idiopathic hypoglycemia and insuloma in children would be greatly simplified.

When the differentiation between functional hypoglycemia and organic hyperinsulinism presents a clinical problem, it can be solved with a three-hour tolbutamide test without having to resort to starvation for up to seventy-two hours. The tolbutamide test can be performed on an outpatient basis; it conserves time and the expenses of hospitalization. It is of greatest value in patients whose fasting blood sugar levels are in the normal or borderline range, and in whom a diagnosis of a pancreatic insuloma needs to be ruled out. In cases of suspected insulomas with fasting blood sugar levels in the hypoglycemic range, the test can be used for confirmation. However, under those circumstances, it should be performed with great caution. The test should be interrupted by administration of 50 per cent glucose if severe symptoms should occur.

The results of the tolbutamide tests in the patients with functional hyperinsulinism and hypoglycemia associated with diabetes mellitus deserve brief comment. When the patients with functional hyperinsulinism were compared with normal subjects, there was no difference in the hypoglycemic responses to tolbutamide. This indicates that subjects with functional hyperinsulinism do not release an increased amount of insulin following administration of tolbutamide, but it seems probable that they do following administration of glucose (28).

In patients with mild diabetes mellitus and secondary hypoglycemia, a delayed release of insulin after administration of glucose has been postulated (11). The initial absorptive hyperglycemia has been thought to afford a supernormal stimulus to the islets of Langerhans, resulting in discharge of amounts of insulin sufficient to produce transient hypoglycemia three to five hours after ingestion of glucose. Recent observations of Yalow and Berson with an immunologic assay for insulin support such an interpretation (32). In patients with mild diabetes mellitus, these investigators demonstrated a delay in the normal rapid release of insulin after administration of glucose, followed by a supernormal release of insulin. In the majority of our patients with diabetes and secondary hypoglycemia, the intravenous tolbutamide test also indicated a delay in the release of insulin, as occurs in more severe diabetes (33).

Addendum

Since submission of this paper we have had the opportunity to perform the tolbutamide test in a patient with untreated panhypopituitarism due to postpartum infarction of the pituitary. Severe pharyngitis developed and the patient was unable to eat for a

period of thirty-six hours. She was admitted to University Hospital in a semicomatose state. The blood sugar concentration was between 20 and 30 mg. per 100 ml. After an adequate nutritional intake for several days, but before institution of hormonal therapy, the levels of fasting blood sugar were in the normal range. Results of the tolbutamide test were as follows: fasting blood sugar level, 79 mg. per 100 ml.; fifteen minutes, 70 mg.; thirty minutes, 46 mg.; forty-five minutes, 38 mg.; one hour, 37 mg.; one and a half hours, 50 mg.; two hours 60 mg.; two and a half hours, 59 mg.; and three hours, 59 mg. per 100 ml.

We have learned (Williams, R. H., personal communication) about another patient with a proven insuloma who showed the same characteristic response to intravenous tolbutamide as seen in the other 16 cases mentioned. One patient was found to have a malignant insuloma with metastases (15). In contradistinction to these 17 cases, we have heard (Waters, C. M. and Van Wyk, J. J., personal communication) about a 15-year-old boy with a proven insuloma in whom there was a nondiagnostic response to tolbutamide.

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INFLUENCE OF THYROID FUNCTION ON CARBOHYDRATE METABOLISM AND A NEW METHOD FOR ASSESSING RESPONSE TO INSULIN

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ABSTRACT

A new method for assessing both glucose utilization and response to insulin is presented. The technique eliminates certain shortcomings of previous methods, which have yielded widely varying results in patients with abnormal thyroid function. The results of the application of the new procedure to 38 euthyroid patients, 15 patients with hyperthyroidism and 13 patients with hypothyroidism, are presented. In the euthyroid group, the mean specific rate constant for glucose utilization (K) was .0349; for the hyperthyroid patients it was .0325; and for the hypothyroid patients it was .0269. The mean K value for the hypothyroid patients was significantly less than that for the euthyroid patients, whereas the mean K value for the hyperthyroid patients was not significantly different from that for the euthyroid patients. In the euthyroid group, insulin resulted in a mean increase in K of 59 per cent. The hyperthyroid patients showed a significantly greater response (+104 per cent) to insulin. The hypothyroid patients showed an insignificant response (+18 per cent) to insulin. The interpretation of these results is discussed with regard to other data on the relationship of thyroid function to carbohydrate metabolism.

ALTHOUGH it is well known that disturbances of thyroid function in man have a pronounced influence on carbohydrate metabolism, the nature of this influence is uncertain. It is generally believed that hyperthyroidism increases the insulin requirement in diabetic patients and that myxedema has the opposite effect (1). There is experimental evidence that thyroxine has a diabetogenic action and that thyroidectomy inhibits the development of diabetes (2). However, the mechanism of these effects is obscured by the marked variability in the reported findings. Normal (3, 4), increased (5, 6), decreased (7–10), or highly variable (11) glucose tolerance or utilization have been described in patients and animals with hyperthyroidism. Insulin sensitivity or tolerance has been reported to be normal (9, 12), decreased (13–15) or increased (16) in thyrotoxicosis. The same

Received August 5, 1960.

confusion is found in the literature on hypothyroid patients and animals (3, 11, 16–22).

Much of this disagreement is due to shortcomings of the techniques used to measure glucose tolerance and insulin response. A critical appraisal of the numerous techniques reveals that all suffer from one or more important weaknesses, in that they do not allow for: (*a*) the intestinal absorption of glucose (3, 4, 6, 8, 9, 17, 18, 23); (*b*) the occurrence of hypoglycemia (19–22, 24–27); (*c*) the occurrence of excessive hyperglycemia and glycosuria (18, 28); (*d*) the use of incorrect or insensitive methods of data analysis (7, 9–12, 18, 24–26, 28, 29); (*e*) the use of relatively large doses of insulin (20, 24, 29); (*f*) failure to differentiate between insulin sensitivity and response to hypoglycemia (20, 22, 26); and (*g*) the variations in the responses to glucose and insulin in tests performed on different days (9, 11, 12, 21, 23, 25). Any one of these factors could result in erroneous interpretations.

In an effort to clarify the relationship between thyroid function and carbohydrate metabolism we have developed a new method for assessing both glucose utilization and insulin sensitivity, specifically designed to eliminate the cited shortcomings of previous methods. The purpose of this paper is to describe the new method and to report results of its application in euthyroid patients and in patients with abnormal thyroid function.

METHOD

Patients

The 38 (37 male and 1 female) euthyroid subjects were in good nutritional and general condition, convalescing uneventfully in the hospital from nonendocrine or acute illnesses. The 28 (24 male and 4 female) patients with thyroid disease were those in whom the diagnosis could be made from clinical and laboratory findings. The hyperthyroid group consisted of 14 cases of Graves' disease (moderate or severe) and 1 case of asymptomatic goiter made moderately thyrotoxic by administration of desiccated thyroid. The hypothyroid group consisted of 5 cases of idiopathic myxedema and 8 of hypothyroidism secondary to therapy (I^{131} or surgery) for hyperthyroidism or heart disease. All patients received the regular hospital diet for at least three days before being studied. All studies were performed in a special air conditioned room between 8:00 and 10:00 a.m., after a fourteen-hour fast and following a ninety-minute rest period in the test bed.

Test procedure

A 10 per cent aqueous solution of glucose was infused intravenously into the antecubital vein for sixty minutes at a constant rate in each patient, by means of a Bowman constant infusion pump. In all patients, a dose of 3 units of glucagon-free insulin[1] was injected intravenously immediately following the infusion. The insulin solution (4 units per ml.) for injection was prepared freshly every five days by diluting stock insulin (40 units per ml.) with sterile water. The diluted insulin was checked for adsorption to

[1] Obtained from Eli Lilly and Co., Indianapolis, through the courtesy of Dr. W. R. Kirtley.

glass, using I^{131}-insulin;[2] the adsorption was found to be less than 5 per cent at the insulin concentration used. Capillary blood samples (0.2 ml.) were obtained from the finger (patient's hand warmed by an electric heating pad) just before the start of the infusion and thereafter at intervals of five or ten minutes for a period of 100–120 minutes. The blood was analyzed for glucose by the Somogyi-Nelson method (30). Care was taken to avoid glycolysis by using sodium fluoride and immediately refrigerating the samples. All analyses were completed within five hours of the end of each study. Urine was collected at the end of some studies and analyzed for glucose content by the Somogyi-Nelson method. In the euthyroid group, glucose excretion was negligible (mean <1 per cent of administered glucose). Because the highest blood sugar levels in the patients with thyroid disease were virtually identical with those in the euthyroid group, urinary glucose measurements were not made in the thyroid patients.

Rationale of method

We have previously demonstrated that the continuous intravenous glucose infusion technique and the slope analysis method for analyzing the resulting data yield a valid index of carbohydrate metabolism (31). Furthermore, we showed that the blood glucose disappearance rate constant[3] (K) during a continuous intravenous glucose infusion is statistically identical to that constant immediately following the infusion. Thus, when insulin is administered at the end of the glucose infusion, the difference between the two rate constants represents the influence of insulin on glucose disappearance.

For clarity, a brief summary of the method and the equations employed is presented. During the continuous infusion of glucose the behavior of blood glucose is described by the following equation:

$$C_g = (C_f - C_{eq}')e^{-Kt} + C_{eq}' \qquad \text{I}$$

where

C_g = arterial blood glucose concentration at any time (t).
C_{eq}' = theoretical blood glucose concentration at equilibrium ($t = \infty$).
C_f = fasting blood glucose concentration.
K = specific rate constant for glucose utilization.

After the glucose infusion, the behavior of blood glucose is described by the following equation:

$$C_g = (C_0 - C_{eq})e^{-Kt} + C_{eq} \qquad \text{II}$$

where

C_{eq} = theoretical blood glucose concentration at equilibrium ($t = \infty$).
C_0 = theoretical maximal blood glucose concentration at the end of the glucose infusion.

The first derivatives of these equations with respect to time represent the slopes of the blood glucose curves under these two experimental conditions:

$$\frac{dC_g}{dt} = -KC_g + KC_{eq}' \qquad \text{IA}$$

[2] Obtained from Abbott Laboratories, North Chicago, Illinois.

[3] The specific rate constant, K, is a proportionality constant defined as the rate of glucose utilization when the blood glucose concentration is equal to unity. Glucose utilization is defined here as the disappearance of blood glucose.

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$$\frac{dC_g}{dt} = -KC_g + KC_{eq} \qquad \text{IIA.}$$

Thus, the rate constant (K) is obtained from a plot of the slope of the blood glucose-time curve versus blood glucose concentration. The slope of the blood glucose-time curve is measured directly by means of a mechanical slope reader (31). In the continuous infusion studies, the volume of distribution of glucose (V) is calculated from the following equation (32):

$$V = \frac{R_i C_i}{K(C_{eq}' - C_f)}$$

where

R_i = rate of infusion.
C_i = concentration of glucose in infused fluid.

RESULTS

Euthyroid patients

Table 1 contains data from the 38 euthyroid patients who received a constant intravenous infusion of glucose for sixty minutes followed by the rapid intravenous administration of 3 units of regular insulin. In Figure 1 the data on one of these subjects (C.R.) are plotted. For comparative purposes, Figure 1 also contains data on a normal subject (F.T.) who received glucose only.

In the 38 euthyroid patients, C_f exceeded C_{eq} without exception. The mean C_{eq} (40 mg. per 100 ml.) was 56 per cent below the mean C_f (90 mg. per 100 ml.), and this difference was highly significant ($P < .001$). K_2 also exceeded K_1 without exception. The mean difference between K_1 and K_2 was 59 per cent, and this difference was also highly significant ($P < .001$). The mean volume of distribution of glucose (V) was 21.3 per cent of body weight. The 27 subjects who were less than 50 years old showed a greater response to insulin, as manifested by a greater decrease in C_{eq} and a larger increase in K, but these differences were not significant ($P > .15$).

Patients with hyperthyroidism

Table 2 contains data on the 15 patients with hyperthyroidism who received the sixty-minute constant intravenous glucose infusion followed by 3 units of insulin intravenously. Figure 2 shows the data on one of these patients (H.W.).

The mean C_f (95 mg. per 100 ml.) was significantly ($P < .01$) higher than the mean C_f (90 mg. per 100 ml.) for the euthyroid group. The difference between C_f and C_{eq}, although highly significant ($P < .001$), was not significantly ($P = .19$) greater than the corresponding difference observed in the euthyroid patients. The mean K_1 (.0325) was slightly lower than the mean K_1 (.0349) of the euthyroid group, but this difference was not significant

TABLE 1. EUTHYROID PATIENTS*

Patient				Rate (mg./ min.)	C_f (mg./ 100 ml.)	C_{eq} (mg./ 100 ml.)	C_{eq}' (mg./ 100 ml.)	K_1 (min.$^{-1}$)	K_2 (min.$^{-1}$)	V (% of body wt.)
Initials	Age (yrs.)	Height (ins.)	Weight (Kg.)							
M.M.	22	72	73	300	85	4	126	.0488	.0490	21.7
R.St.	26	69	73	372	89	26	172	.0365	.0442	17.2
E.F.	27	70	66	366	82	24	167	.0315	.0692	20.5
A.D.	30	69	53	352	87	29	150	.0463	.0593	23.6
H.K.	31	76	88	330	86	52	173	.0185	.0336	24.1
J.W.	31	68	60	304	85	28	131	.0423	.0608	28.5
R.B.	32	70	70	322	93	33	133	.0535	.0850	21.5
G.D.	32	69	64	334	101	36	168	.0437	.0640	18.5
J.B.	33	72	73	329	102	11	161	.0408	.0690	20.9
I.S.	33	69	73	357	92	25	154	.0361	.0433	23.8
D.D.	34	78	97	314	85	55	140	.0260	.0427	23.5
R.S.	35	69	61	344	91	40	185	.0310	.0454	19.8
E.T.	35	67	61	373	91	83	178	.0288	.0458	24.5
R.C.	36	76	86	348	88	62	146	.0352	.0664	21.3
E.G.	37	66	71	340	87	24	143	.0429	.1008	19.5
A.W.	37	71	79	372	81	66	167	.0229	.0910	25.0
W.J.	38	66	63	340	85	52	169	.0366	.0424	17.7
C.V.	40	75	78	350	84	32	131	.0509	.0592	19.7
O.M.	40	64	68	352	95	45	191	.0275	.0574	20.6
T.W.	41	71	66	307	81	47	140	.0320	.0572	25.5
F.P.	42	71	76	382	94	29	161	.0420	.0691	18.3
E.Q.	43	68	74	364	82	39	180	.0212	.0356	24.6
G.W.	44	69	70	352	92	21	176	.0362	.0560	18.8
R.R.	46	70	91	360	95	74	159	.0212	.0280	26.3
G.A.	48	71	76	332	95	14	157	.0378	.0422	18.5
J.D.	49	69	61	372	88	17	168	.0357	.0547	24.4
L.R.†	49	67	57	320	95	25	177	.0376	.0594	18.5
R.M.	52	66	75	338	82	29	177	.0216	.0411	22.4
B.W.	53	68	61	356	87	17	163	.0383	.0531	19.8
J.J.	61	69	77	392	96	48	180	.0359	.0602	18.0
J.S.	62	72	67	332	93	14	151	.0426	.0471	21.1
C.K.	63	74	89	330	92	60	142	.0499	.0586	14.9
W.Z.	64	61	61	354	89	56	165	.0342	.0467	22.3
C.B.	67	68	62	338	90	28	205	.0199	.0376	23.7
L.K.	68	64	76	340	87	70	172	.0303	.0312	19.5
C.R.	69	71	75	332	94	79	150	.0349	.0548	22.3
W.R.	70	68	79	350	90	67	173	.0267	.0374	20.3
W.B.	78	66	61	343	97	52	198	.0279	.0419	21.2
Mean					90	40		.0349	.0537	21.3
Std. Dev.					5	20		.0087	.0157	2.8

* Definition of symbols:
Rate = rate of glucose infusion;
C_f = fasting blood glucose concentration;
C_{eq}, C_{eq}' = theoretical blood glucose concentrations at equilibrium;
K_1 = specific rate constant for glucose utilization during glucose infusion;
K_2 = specific rate constant for glucose utilization after glucose infusion and insulin injection;
V = volume of distribution of glucose.
† The only female among the 38 patients.

($P = .31$). The difference between K_2 and K_1 was highly significant ($P < .001$) and this difference (representing a 104 per cent increase in K) was significantly ($P < .01$) greater than the corresponding difference (59 per cent) observed in the euthyroid group. It should be noted that whereas in the euthyroid patients insulin caused a decrease in C_{eq} and an increase in K of equal magnitude, in the hyperthyroid patients insulin caused a similar decrease in C_{eq} but an increase in K that was almost twice as great as in euthyroid patients.

The mean V (25.8 per cent of body weight) was significantly ($P < .001$) greater than the mean V (21.3 per cent) for the euthyroid group. Since this may have been an artifact of weight loss in the hyperthyroid patient, these volumes were recalculated using ideal weights as a reference. Although the

mean value (24.2 per cent) calculated in this manner was slightly lower than the actual value, it was still significantly ($P < .001$) higher than the corresponding V for the euthyroid group.

Patients with hypothyroidism

Table 3 shows data on the 13 patients with hypothyroidism who received 3 units of insulin at the end of the constant intravenous infusion of glucose. Figure 3 is a plot of the data on one of these patients.

The mean C_f (84 mg. per 100 ml.) was significantly ($P < .01$) below the

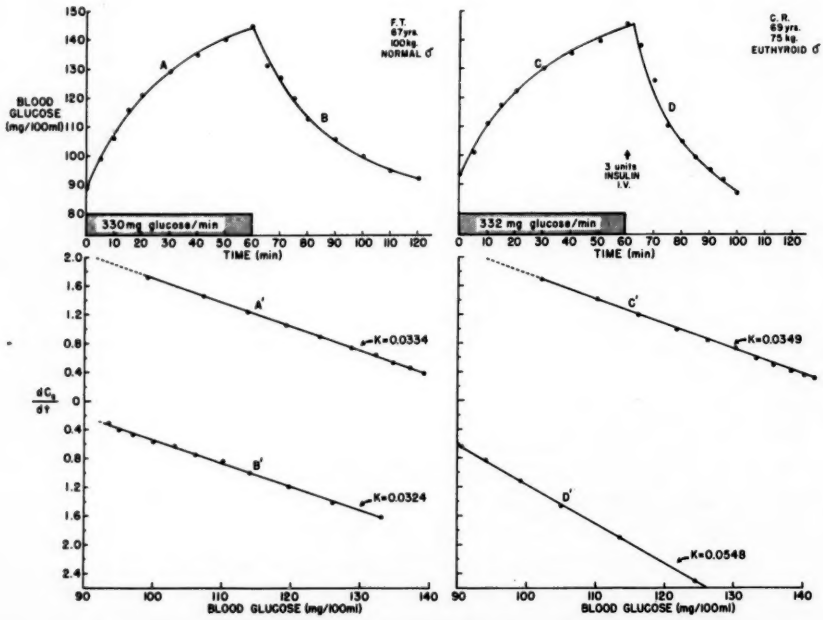

FIG. 1. Comparison of data on a patient from the euthyroid group (glucose followed by insulin) with data on a normal subject (glucose only).

F.T. was a normal subject who received an infusion of glucose for sixty minutes. The upper left graph is a plot of blood glucose concentration *vs.* time; curve A was obtained during the infusion (equation I) and curve B was obtained following the infusion (equation II). The analysis by the slope method is shown in the lower left corner; line A′ represents the analysis of curve A (equation IA) and line B′ represents the analysis of curve B (equation IIA). Note the approximate equality of K values.

C.R. was a euthyroid patient who received insulin at the end of the 60-minute infusion of glucose. In the upper right graph, curve C was obtained during the infusion and curve D after the infusion and injection of insulin. The analysis is shown in the lower right corner. Note that the K obtained from D′ was 57 per cent greater than that from C′; the increase in K represents the effect of insulin.

TABLE 2. HYPERTHYROID PATIENTS*

Patients				Rate (mg./min.)	C_f (mg./100 ml.)	C_{eq} (mg./100 ml.)	C_{eq}' (mg./100 ml.)	K_1 (min.$^{-1}$)	K_2 (min.$^{-1}$)	V (% of body wt.)
Initials	Age (yrs.)	Height (ins.)	Weight (Kg.)							
A.E.	27	64	54	326	82	19	141	.0354	.0675	29.4
J.F.	34	67	60	380	94	31	182	.0278	.0582	26.5
W.H.	63	66	56	342	94	21	163	.0290	.0502	30.5
C.G.	64	69	56	346	98	19	195	.0221	.0316	28.5
H.W.†	53	61	47	338	100	44	172	.0399	.0990	27.6
W.R.	58	72	74	336	96	56	167	.0220	.0604	29.5
P.L.	62	68	67	374	98	52	188	.0274	.0471	23.5
H.We.	39	70	75	350	103	48	154	.0344	.0774	29.7
R.S.	26	70	78	363	90	20	152	.0424	.0457	20.3
P.S.	66	66	54	340	109	41	193	.0374	.0668	20.9
E.M.	59	76	101	334	99	78	145	.0337	.0514	21.8
D.E.†	36	65	69	330	99	48	156	.0436	.1328	19.4
C.S.†	58	62	48	328	86	29	203	.0310	.0636	19.1
J.T.	45	70	80	342	92	28	140	.0301	.0636	29.8
C.B.	33	69	45	302	85	32	156	.0308	.0596	30.6
Mean					95	38		.0325	.0650	25.8
Std. Dev.					7	17		.0065	.0242	4.5

* For definition of symbols, see footnote to Table 1.
† Female.

mean C_f (90 mg. per 100 ml.) for the euthyroid group. The difference between C_f and C_{eq}, although highly significant ($P < .001$), was not significantly ($P = .08$) less than the corresponding difference observed in euthyroid patients. It should be noted that the C_{eq} values for 2 patients (C.M.

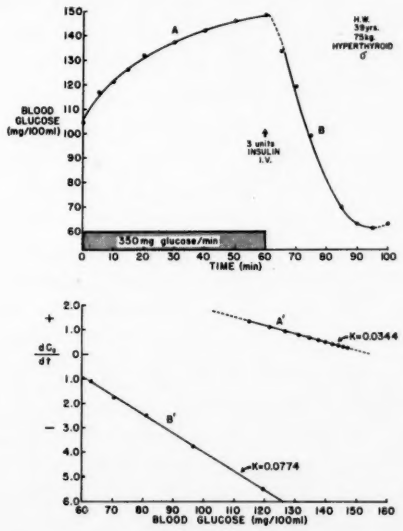

FIG. 2. Data on a patient with hyperthyroidism.

The upper graph is a plot of blood glucose concentration *vs.* time; curve A was obtained during the infusion of glucose, and curve B after the infusion of glucose and injection of insulin. In the lower graph, lines A′ and B′ represent the analyses of curves A and B respectively. The K value after insulin was 125 per cent greater than that before insulin.

TABLE 3. HYPOTHYROID PATIENTS*

Patient					Rate (mg./min.)	C_f (mg./100 ml.)	C_{eq} (mg./100 ml.)	C_{eq}' (mg./100 ml.)	K_1 (min.$^{-1}$)	K_2 (min.$^{-1}$)	V (% of body wt.)
Initials	Diagnosis**	Age (yrs.)	Height (ins.)	Weight (Kg.)							
F.C.	I	65	72	72	326	101	64	201	.0230	.0272	17.0
E.G.	I	65	69	94	344	95	28	180	.0208	.0231	22.4
O.R.	I	40	66	58	310	81	37	172	.0339	.0187	17.5
I.S.	I	33	68	62	332	86	55	212	.0165	.0255	25.6
M.C.	I	61	70	77	304	77	85	149	.0270	.0634	20.0
L.D.	S	64	63	60	354	82	49	178	.0263	.0108	24.0
R.K.†	S	56	65	62	362	83	21	203	.0235	.0205	21.9
C.M.	S	23	68	76	330	81	—‡	142	.0436	.0083	19.3
K.S.	S	25	66	66	314	83	23	149	.0346	.0392	20.9
J.W.	I^{131}	75	71	61	340	83	65	178	.0223	.0213	29.2
E.J.	I^{131}	47	66	73	344	68	10	112	.0420	.0552	25.4
P.L.	I^{131}	62	68	64	330	86	—‡	190	.0115	.0290	29.1
L.P.	I^{131}	66	68	59	360	87	89	187	.0253	.0320	26.8
Mean						84	48		.0269	.0288	23.0
Std. Dev.						8	26		.0093	.0152	3.9

* For definition of symbols, see footnote to Table 1.
** I = idiopathic myxedema; S = post-surgical myxedema; I^{131} = myxedema following I^{131} therapy.
† Female.
‡ C_{eq} values for Patients C.M. and P.L. were negative (see text) and are not included in the table.

and P.L.) in this group were negative, and that this did not occur in any of the euthyroid or hyperthyroid patients. The mean K_1 (.0269) was significantly ($P < .01$) lower than the corresponding value for the euthyroid group. The mean difference between K_1 and K_2 was not significant ($P = .70$). The mean V (23.0 per cent of body weight) was not significantly ($P = .11$) higher than the mean V of the euthyroid group. When V was recalculated on the basis of an ideal weight, the new mean value was 23.1 per cent—virtually identical to the actual value.

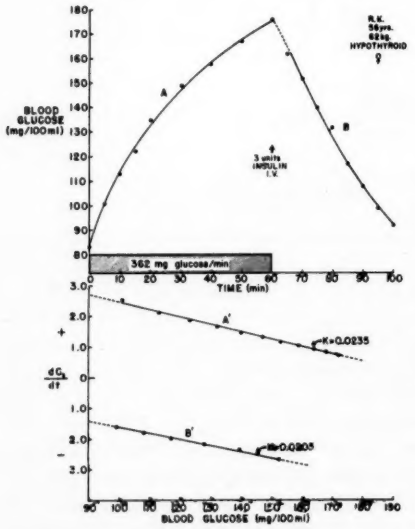

FIG. 3. Data on a patient with hypothyroidism. The upper graph is a plot of blood glucose concentration *vs.* time; curve A was obtained during the infusion of glucose, and curve B after the infusion of glucose and injection of insulin. In the lower graph, lines A′ and B′ represent the analyses of curves A and B respectively. The K value after insulin was 13 per cent less than that before insulin.

DISCUSSION

The method

We have previously shown that the blood glucose disappearance rate constant (K) obtained during a constant intravenous infusion of glucose is the same as that constant obtained after the infusion (31). Thus, if a given compound is administered at the end of the glucose infusion, the first K serves as a control value and the second K represents the effect of the compound on glucose utilization. This principle has been used to assess insulin response as well as glucose utilization.

We have sought to eliminate the shortcomings of previous techniques cited in the introduction. In order to cause a minimum disturbance of physiologic conditions, glucose was administered intravenously at a slow constant rate (32); the sudden load of glucose presented to the system in the single-injection method and the resulting excessive hyperglycemia were thus avoided. The maximal blood sugar level (mean) in all patients was only 155 mg. per 100 ml. and glycosuria sufficient to complicate the calculation of K was not observed (33). The entire test was completed within two hours, thus avoiding the variations in carbohydrate metabolism which occur from day to day. A small fixed dose of insulin was used to increase the sensitivity of the method, because there is no evidence that insulin action is related to body weight (34). The small dose of insulin and the infusion of glucose before administration of insulin prevented hypoglycemia and the initiation of counter-regulatory mechanisms. Finally, the method of data analysis used yields an index of glucose utilization which is independent of the exponential equation assumed to describe blood glucose disappearance, whereas previous methods employed incorrect equations or insensitive methods of data analysis or both (31). For these reasons, the present method is considered to yield more valid measures of insulin sensitivity and glucose utilization than previous methods.

Euthyroid patients

We have previously found that in normal subjects C_{eq} was 21 per cent less than C_f following a constant intravenous infusion of glucose (31). In the present studies, insulin caused a significant ($P < .001$) increase in this difference (56 per cent). Similarly, the difference in K values (59 per cent) with insulin was significantly ($P < .001$) greater than the corresponding difference (5 per cent) previously observed without insulin (31). Thus, we have shown that insulin has two consistent effects: it lowers the C_{eq} value and it increases the K value. Either one of these effects may serve as an index of insulin responsiveness since the changes observed were large and equal in magnitude. However, the equality of these changes is coincidental,

since C_{eq} and K are theoretically (31) and experimentally (see next section) independent. The C_{eq} is a reference level indicating the blood glucose concentration at which the net hepatic output of glucose is equal to the net peripheral glucose utilization. Thus the lowered C_{eq} as well as the increased K noted after insulin in normal subjects is consistent with the known actions of insulin, namely, to increase hepatic uptake and peripheral utilization of glucose.

Thyroid function in relation to carbohydrate metabolism

It would be difficult to predict from previous experimental data whether glucose utilization would be increased, decreased, or normal in thyrotoxicosis. Peripheral tissues from animals treated with large doses of thyroxine show increased oxygen consumption and increased glucose utilization (35). An increased metabolic rate *per se* would be expected to increase glucose utilization; indeed, an increase in glucose utilization (36, 37) and in K values (38) following physical activity has been observed in the normal and in the diabetic human.

On the other hand, there is evidence which suggests that thyroid hormone accelerates the degradation of insulin in the rat (39, 40) and our preliminary studies indicate that thyroid function also influences the degradation of insulin in man (41). An increased rate of insulin degradation without a comparable increase in the rate of insulin production in the thyrotoxic patient would result in subnormal amounts of available insulin and a decreased rate of glucose utilization. This phenomenon could explain the diabetogenic action of thyroxine in the partially depancreatized dog (2) and the increased insulin requirement of the human diabetic in whom thyrotoxicosis develops (1).

Our findings of normal K values and normal C_r–C_{eq} differences in thyrotoxic patients suggest that these patients utilize glucose at a normal rate. There are at least two possible explanations for this observation. The specific rate constant for peripheral glucose utilization is directly proportional to arterial-venous glucose differences and peripheral blood flow, whereas it is inversely proportional to the volume of distribution of glucose (32). In hyperthyroidism, peripheral blood flow is probably increased and our data reveal a significantly increased volume of distribution of glucose. If these changes were proportional, no change in K would occur. The second possible explanation for the normal K values in our patients with hyperthyroidism is that the other factors (basal oxygen consumption, insulin degradation) which influence the rate of glucose utilization in opposite directions cancel each other out. The same factors are probably operative in the hypothyroid patient, but the effects are reversed. Hypoactivity lowers glucose tolerance (42), and hypothyroidism would be expected to

decrease insulin production and degradation. Because we found a significantly decreased K value in the hypothyroid patients, it would appear that the influence of hypoactivity and decreased insulin production (which would decrease K) predominate over the factor of decreased insulin degradation (which would increase K).

Our findings of an elevated fasting blood sugar (FBS) level in hyperthyroid patients and a decreased level in the hypothyroid patients confirm previous reports (1, 6). The elevated FBS level in the hyperthyroid patients could be due to an increased breakdown of liver glycogen (43) or to an increase in insulin degradation without a comparable increase in insulin production, or both. The low FBS level in the hypothyroid patient, is more difficult to explain. It is probably the result of a complex interplay of a number of factors such as hypoactivity, reduced hepatic glucose output, and diminished adrenal function.

In the hyperthyroid patients, insulin resulted in a mean C_{eq} value which was 60 per cent below the mean C_f; in the euthyroid group the corresponding value was 56 per cent, and in the hypothyroid group it was 43 per cent. This trend indicates a difference in insulin sensitivity in the 3 groups of patients, but these differences are not statistically significant. It is noteworthy that following administration of insulin, 2 of the 13 hypothyroid patients had C_{eq} values below zero, whereas all of the 38 euthyroid and 15 hyperthyroid patients had C_{eq} values above zero. We have also observed negative C_{eq} values following administration of insulin in 7 of 32 patients with parenchymatous liver disease (41). Furthermore, in 14 of these 32 patients there was no increase in K value after administration of insulin, and the 7 patients with negative C_{eq} values were all among the 14 who did not respond to insulin. A negative C_{eq} value is theoretically due to increased peripheral glucose utilization or decreased net hepatic glucose output, or both. There is evidence that, following administration of insulin, peripheral glucose utilization is not increased (or actually decreased) in some patients with liver disease (41) and hypothyroidism. It is therefore probable that the negative C_{eq} values in these two groups of patients were due largely to a decreased net hepatic output of glucose without an increase (or with an actual decrease) in peripheral glucose utilization.

Marked differences in insulin sensitivity were manifested by changes in K values after injection of insulin. In the hyperthyroid patients the mean K value following insulin was 104 per cent greater than the control; in the euthyroid group it was 59 per cent greater, and in the hypothyroid patients it was only 18 per cent greater. The changes in K value following administration of insulin in the patients with thyroid disease were significantly different from the changes noted in the euthyroid patients, and thus constituted a sensitive index of the effect of insulin.

The increased sensitivity to insulin in the thyrotoxic patient demonstrated with our procedure appears to be inconsistent with the clinical and experimental evidence for a diabetogenic action of excess thyroid hormone and with our stated postulation of the mechanism of this action. The explanation of this paradox probably lies in the method of insulin administration. The rapid intravenous injection of insulin used in our procedure quickly initiates an action on blood sugar and this effect could easily obscure the influence of an increased rate of insulin degradation on blood sugar. In contrast, insulin administered by the usual therapeutic route (subcutaneously) causes a slow fall in the blood sugar level and therefore the effects of an altered rate of insulin degradation would be much more evident.

In the hypothyroid patient the response to insulin was decreased. It is suggested that this is a manifestation of the sluggish end-organ response characteristic of the disease. The factor of a decreased rate of insulin degradation in hypothyroidism is obscured by the effect of rapid intravenous injection of insulin, as postulated for the hyperthyroid patient.

The expanded volume of distribution of glucose in the hyperthyroid patients cannot be explained by a decreased body weight shared equally by fat and lean body mass. It could be due to a loss of body fat (low fluid content) proportionately greater than a loss of lean body mass. Lean body mass is significantly decreased in thyrotoxicosis, and the reverse is true in myxedema (44). In the hypothyroid patients the slightly elevated volume could be attributed to the edema characteristic of the disease.

Acknowledgment

The authors wish to thank Mr. A. Fiskin for his many valuable suggestions in the preparation of this manuscript.

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JCEM

STUDIES ON PATIENTS WITH ISLET-CELL TUMOR, INCLUDING THE PHENOMENON OF LEUCINE-INDUCED ACCENTUATION OF HYPOGLYCEMIA*

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ABSTRACT

Three patients with islet-cell tumor of the pancreas are presented. All had severe fasting hypoglycemia relieved by the administration of glucose. However, 1 had a normal intravenous tolerance curve and 2 did not show any sensitivity to intravenous glucagon-free insulin. The effects of leucine and glycine on the blood sugar level were studied. Administration of leucine consistently caused accentuation of the tumor-induced hypoglycemia—the fall ranging from 21 to 76 per cent below the fasting level—accompanied by clinical symptoms, whereas administration of glycine caused no appreciable changes. Following extirpation of the tumors, leucine provoked no significant degree of hypoglycemia. In 2 normal subjects and in 5 patients with functional hypoglycemia, administration of leucine caused no changes in the blood sugar levels. The data favor the view that, in cases of islet-cell tumor, leucine acts by potentiating the peripheral action of insulin.

THE effect of the administration of proteins and amino acids on blood sugar levels has been the subject of considerable investigation (1) and some controversy (2). Recently, Cochrane *et al.* (2) demonstrated a dramatic accentuation of hypoglycemia following the feeding of casein or leucine[1] to some, but not all (3, 4) children with idiopathic hypoglycemia. Since the mechanism of this action of leucine has not been clearly elucidated we thought that useful information might be gained by ascertaining the effect of leucine on the blood sugar levels of patients with islet-cell tumors. To date, 3 such patients have been studied, 2 of whom have been subjects of a report published in abstract (5). The results obtained form

Received July 13, 1960.

* Supported, in part, by research grants from the Wilhelmina Rice Foundation and the National Institute of Arthritis and Metabolic Diseases.

Presented in part, at the Thirty-Second Meeting of the Central Society for Clinical Research, November, 1959.

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[1] Throughout this paper L-leucine is designated as "leucine."

the basis of this presentation; also included are data regarding the effect of administration of leucine in normal subjects and patients with functional hypoglycemia.

CASE REPORTS

Case 1

P.E. (#629215), a 39-year-old white housewife, was admitted to Presbyterian-St. Luke's Hospital on March 20, 1959, for investigation of an episode of bizarre behavior that had occurred one week prior to admission. At that time she was found in bed at 7:00 a.m., restless, confused and exhibiting erratic hyperkinetic activity. The attack lasted about three hours and seemed to be terminated by taking food. This was the first such episode but it is of interest that a glucose tolerance test performed nine years previously during her third pregnancy showed a rather flat curve: fasting blood sugar was 75 mg.; half hour, 91 mg.; one hour, 105 mg.; two hours, 110 mg.; and three hours, 103 mg. per 100 ml. A high-protein diet was prescribed but she did not recall any detrimental effects that could be attributed to it. The family history revealed that the paternal grandmother had diabetes mellitus. There was no history of convulsive disorders. The patient's weight had remained constant and there had been no recent change in dietary habits.

Physical examination showed a thin but well developed white female who was well oriented and cooperative. The blood pressure was 106/70 mm. Hg, and the pulse was 92. The liver edge was palpable at 3 cm. below the right costal margin, and was slightly tender. The findings on neurologic examination were entirely normal.

At the time of admission the hemoglobin level was 13.0 Gm. per cent, and the white blood cell count was 9,200 per cu. mm. with a normal differential count. Urinalysis findings were entirely normal. The level of blood urea nitrogen was 11 mg. per 100 ml., serum calcium 9 mg. per 100 ml., protein-bound iodine 4.2 μg. per 100 ml., and erythrocyte uptake of I^{131}-triiodothyronine 13.8 per cent (normal range, 9 to 16 per cent). Liver function tests revealed a serum total protein level of 7.2 Gm. per cent, with albumin 5.2 Gm. per cent. There was 7 per cent and 3.6 per cent retention of bromsulfonphthalein on two occasions. Serum alkaline phosphatase activity was 4.1 Bodansky units, serum amylase activity 39 units, and thymol turbidity 5.9 units. Roentgenograms of the chest, skull, esophagus, stomach and duodenum were normal. The initial 24-hour urinary excretion of 17-hydroxycorticosteroids was 17.9 mg. (normal, 3–10 mg.), whereas that of 17-ketosteroids was 11.0 mg. (normal, 6–12 mg.); however, later determinations were within the normal range.

Fasting blood sugar levels were 23 mg. and 20 mg. per 100 ml., but symptoms were present only at the time of the 20-mg. value. The patient responded promptly to oral glucose. The hepatic response to epinephrine (0.5 mg. intramuscularly) was considered normal, with a fasting blood glucose level of 62 mg. per 100 ml., rising to 111 mg. per 100 ml. in one hour. The results of subsequent preoperative studies are described later.

On the twenty-fourth hospital day, after supportive adrenal steroid therapy was begun, a solitary encapsulated tumor was removed surgically from the mid-portion of the pancreas. The pancreas was otherwise apparently normal. The histologic diagnosis on the surgical specimen was islet-cell adenoma. Postoperatively, the patient's course was benign, except for temporary drainage from the area of the incision. Repeated determinations of the fasting blood sugar level after withdrawal of corticosteroid therapy yielded normal values. To date the patient has remained entirely asymptomatic.

Case 2

G.M. (#630064), a 76-year-old white female, entered Presbyterian-St. Luke's Hospital on April 25, 1959, because of episodes of confusion and disorientation. She had been discharged from the hospital only two weeks previously; the diagnosis had been diverticulosis of the colon. At that time a routinely obtained fasting blood sugar determination was normal. In the intervening two weeks she had had four episodes of weakness and confusion. In each instance ingestion of food seemed to improve her condition. There was no past history of diabetes mellitus, liver disease or nervous disorders.

Physical examination revealed an alert, oriented white elderly female. The blood pressure was 140/80 mm. Hg and the pulse was 80. A soft systolic murmur was noted in the aortic area. The rest of the physical findings were normal.

Laboratory studies showed a hemoglobin level of 12.4 Gm. per cent, and a white blood cell count of 5,350 per cu. mm. with a normal differential count. The results of urinalysis were entirely normal. The level of blood urea nitrogen was 14 mg. per 100 ml., serum sodium 135 mEq. per liter, potassium 4.6 mEq. per liter, calcium 9.1 mg. per 100 ml., and phosphorus 3.8 mg. per 100 ml. Liver function tests showed a bromsulfonphthalein retention of 2.3 per cent, thymol turbidity 0.8 unit, and a serum total bilirubin level of 0.3 mg. per 100 ml. Chest and skull roentgenograms were normal. The 24-hour urinary excretion of steroids was elevated—17-ketosteroids 14.3 mg. and 17-hydroxycorticosteroids, 20.0 mg.

Early on the morning of the second day in the hospital, the patient became disoriented. The blood sugar level at that time was 27 mg. per 100 ml. She responded rapidly to intravenous glucose. Subsequent fasting blood sugar values were 23 mg. and 17 mg. per 100 ml. Results of further studies are described later.

Prednisone therapy (60 mg. per day) was instituted. On the thirteenth hospital day, an exploratory laparotomy was performed. The pancreas was found to be enlarged and firm. Multiple nodules were palpable in the liver, and excisional biopsy of a nodule revealed metastatic islet-cell carcinoma. No further surgery was attempted. Postoperatively the patient was maintained with prednisone, 40 mg. per day. With this therapy, blood sugar values prior to breakfast remained abnormally low. The patient was maintained, subsequently, with doses of prednisone sufficient to prevent further hypoglycemic episodes. Her course was gradually downhill, and she died at home on February 9, 1960.

It is of incidental interest that this patient was given 10 mc. of Zn^{65} intravenously by Dr. Frederic DePeyster, attending surgeon, on May 7, 1959. This was done in order to ascertain whether this isotope would be localized in the tumor (6) and, if so, whether any therapeutic effect could be derived. An open biopsy of a metastatic nodule and surrounding hepatic tissue was performed twelve days later. In addition, sections of the tumor and normal pancreatic tissue were obtained shortly after the patient died. In all specimens, the radioactivity (counts per gram, wet weight) of the uninvolved tissue of liver and pancreas exceeded that found in the tumor. It was concluded by Dr. Frank Hendrickson of the Department of Radiology that no localizing effect could be documented.

Case 3

A.O. (#626692), a 40-year-old white male executive, entered Presbyterian-St. Luke's Hospital on January 18, 1960, complaining of episodes of confusion for the preceding four years. These were relieved by taking food and were usually followed by headache and a feeling of fatigue. The attacks had become more severe in the four months prior

to admission, and on several occasions episodes of unconsciousness had occurred in the early morning hours. In the preceding year he had gained 20 pounds. During previous admissions his blood sugar level had been as low as 40 mg. per 100 ml. after a regimen of fasting and exercise, but no clinical attacks could be precipitated. Past history and family history were not remarkable.

Physical examination revealed a well developed muscular white male whose blood pressure was 134/78 mm. Hg. Bilateral arcus senilis was present but the findings on physical examination were otherwise unremarkable. Laboratory studies revealed a hemoglobin level of 14.3 Gm. per cent, and a white blood cell count of 6,800 per cu. mm. with a normal differential. Results of urinalysis were normal. Bromsulfonphthalein retention at forty-five minutes was 5.1 per cent, and serum alkaline phosphatase activity was 3.8 Bodansky units. The serum protein electrophoresis pattern was normal, and the serum calcium level was 9.5 mg. per 100 ml. The 24-hour excretion of 17-hydroxycorticosteroids was 10.5 mg., and that of 17-ketosteroids was 17.8 mg.

After the further studies described later, the patient was given prednisone, 60 mg. per day, preoperatively. On January 29, 1960, a 2×3 cm. islet-cell adenoma was surgically removed from the tail of the pancreas. Recovery was uneventful and the patient has remained asymptomatic since operation.

As in Patient G.M. (Case 2), the possibility that Zn^{65} would become localized in the islet-cell tumor was explored in this patient. One mc. of Zn^{65} was administered on the day prior to operation, in collaboration with Drs. Frederic DePeyster and Frank Hendrickson. At operation, a collimated probe could not distinguish tumor tissue from normal acinar tissue nor was there any quantitative difference in the counts of radioactivity per gram between the specimens of tumor tissue and normal pancreatic tissue.

PROCEDURE

Blood sugar determinations were initially performed in the clinical chemistry laboratory, using an autoanalyser (Technicon). Later the Nelson modification of the Somogyi technique was employed (7). Since all 3 patients suffered from early morning attacks of clinical hypoglycemia, it was clearly not feasible to carry out studies using the usual Conn preparative procedure (8), which requires determinations in the postabsorptive state. For this reason the patients were given a high-calorie, high-carbohydrate diet with frequent interval feedings, and with particular emphasis on a late evening meal. Using this regimen it became evident that the patients remained free of clinical manifestations of hypoglycemia well through the middle of the morning, until which time breakfast was withheld in order to obtain blood samples for chemical determinations, and to carry out x-ray examinations and other studies.

Venous blood samples were obtained, for the most part, through an indwelling Cournand needle. Leucine or glycine in a dosage of 150 mg. per Kg. of body weight was given (as a suspension in water) by mouth after control blood samples were obtained. The suspensions were well tolerated despite the unpleasant taste of leucine. The intravenous glucose tolerance tests were carried out according to the method of Ikkos and Luft (9). Slopes were derived by visual inspection. The intravenous glucagon-free insulin tolerance test was performed as described in Monaco *et al.* (10).

RESULTS

Intravenous glucose tolerance test

As indicated in Table 1, Patient P.E. responded to the injected glucose with a dramatic fall in blood sugar concentration at a rate (K_G) of 4.6 per

TABLE 1. RESULTS OF INTRAVENOUS GLUCOSE TOLERANCE TESTS IN 3 PATIENTS WITH ISLET-CELL TUMOR

(Fifty ml. of 50 per cent glucose solution was injected over a four-minute period. Multiple venous specimens were obtained through an indwelling Cournand needle.)

Patient	Blood glucose levels (mg./100 ml.)												K_G* (%)
	Control sample	4 mins.	8 mins.	12 mins.	16 mins.	24 mins.	32 mins.	40 mins.	48 mins.	56 mins.	64 mins.	72 mins.	
P.E.	50	295	215	—	170	100	68	50	23	30	30	38	4.6
G.M. #1	50	195	192	175	162	152	127	108	96	86	78	—	1.7
G.M. #2	42	210	195	180	160	148	126	132	126	125	88	80	1.7
	Control sample	10 mins.	15 mins.	20 mins.	25 mins.	30 mins.	35 mins.	40 mins.	45 mins.	50 mins.	55 mins.	60 mins.	
A.O.	24	153	124	115	92	75	85	72	79	72	68	72	3.0**

* K = per cent disappearance of glucose per minute.
** K for Patient A.O. was calculated from samples obtained for only the first 30 minutes following injection of glucose.

cent per minute. In contrast, Patient G.M. showed a normal glucose disappearance rate with a K_G of 1.7 per cent per minute on two occasions. Patient A.O. showed an intermediate and biphasic response, with an initial rapid fall (K_G, 3.0 per cent per minute) followed by a flattening of the curve.

Rapid intravenous insulin tolerance test

Using the criteria suggested (10), this test was performed in 2 patients; one was judged to show no response, and in the other, the response was equivocal. The procedure was carried out on two occasions in Patient P.E. and the results, together with those obtained in Patient A.O., are listed in Table 2. In all three trials there was a measurable fall in blood glucose concentration, in contrast to the insensitivity to insulin found in 2 patients with islet-cell tumor by Monaco *et al.* (10).

Administration of leucine and glycine

The results of these studies in all 3 patients are recorded in Table 3 and shown graphically in Figures 1 and 2. Glycine was selected as a convenient control amino acid for comparing effects with those obtained with leucine.

TABLE 2. RESULTS OF RAPID INTRAVENOUS GLUCAGON-FREE INSULIN TOLERANCE TESTS IN 2 PATIENTS WITH ISLET-CELL TUMOR

(Response in normal controls is reported to be a fall of 19±7 per cent from the fasting level at any time during the six minutes.)

Patient	Blood glucose level (mg./100 ml.)				Maximal % fall from control blood sugar level
	Control	2 mins.	4 mins.	6 mins.	
P.E. #1	35	35	33	26	−26
P.E. #2	55	55	55	46	−16
A.O.	60	56	55	55	− 8

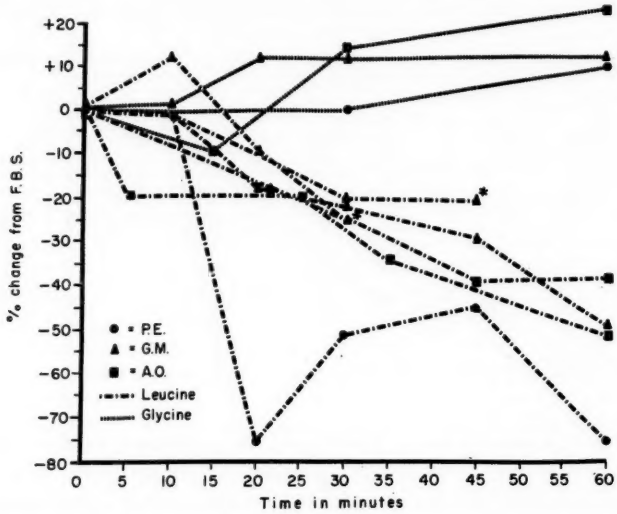

FIG. 1. Effects of leucine compared with glycine on the blood sugar level in 3 patients with islet-cell tumor. The asterisks mark tests discontinued because of severe clinical manifestations of hypoglycemia.

Examination of these data reveal several points of interest. In the presence of hyperfunctioning islet-cell tumor tissue, as reflected by low control blood glucose levels, oral administration of leucine on six occasions uniformly resulted in an appreciable accentuation of hypoglycemia (Fig. 1). The maximal fall in blood sugar concentration occurred between twenty and sixty minutes following administration of leucine; the range of the fall was from 21 to 76 per cent below the fasting level, with a mean of 42 per cent. In an additional instance (Patient A.O.) the intravenous infusion of leucine appeared to exert an effect comparable to that of oral ingestion of leucine, with a maximal fall of 38 per cent in forty-five minutes. In sharp

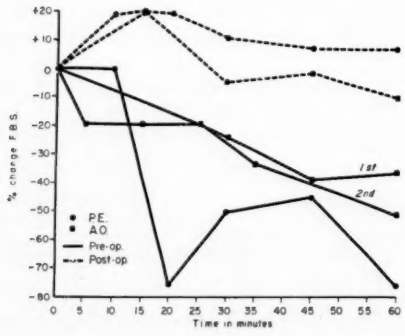

FIG. 2. Effect of removal of islet-cell tumor on blood sugar sensitivity to leucine in Patients P.E. and A.O.

TABLE 3. VENOUS BLOOD SUGAR LEVELS FOLLOWING ADMINISTRATION OF LEUCINE AND GLYCINE IN 3 PATIENTS WITH ISLET-CELL TUMORS

(Changes are expressed as absolute values and maximal percentage changes from the control levels. As indicated, each patient was studied under various conditions during hospitalization.)

Patient	Procedure	Blood sugar level (mg./100 ml.)								Maximal fall or rise	
		Control sample	5 mins.	10 mins.	15 mins.	20 mins.	25 mins.	30 mins.	45 mins.	60 mins.	Absolute (mg./100 ml.)	Per cent of control
P.E.	Pre-op. leucine	21		21		5		10	13	5	−16	−76
	Pre-op. glycine	30		30				30		33	3	10
	Pre-op. leucine +steroids	97		93		93		85			−12	−12
	Pre-op. glycine +steroids	110		106		110		97			−13	−12
	Post-op. leucine	70		83		83		78	75	75	19	27
G.M.	Pre-op. leucine #1	38		43		34		28			−10	−26
	Pre-op. leucine #2	43		43				34	34		− 9	−21
	Pre-op. leucine +steroids	100		105				103	103	97	− 3	− 3
	Pre-op. glycine	38		38		43		43		43	5	13
	Post-op. leucine*	45		45		37			32	23	−22	−49
	Post-op. glycine*	50		43		47			43	50	+ 7	−14
A.O.	Pre-op. leucine #1	43	35		35		35			21	−22	−51
	Pre-op. leucine #2	49						37	30	31	−19	−39
	Pre-op. leucine #3**	34	29	33	28	30	28		21	24	−13	−38
	Pre-op. glycine	30			27			34	35	39	9	30
	Post-op. leucine	91			109			86	89	80	−11	−12

* During these procedures, Patient G.M. was maintained with prednisone, 40 mg. per day, in fractional doses.
** Leucine dissolved in distilled water, in a concentration of 21 mg. per ml., was administered intravenously at a rate of 136 mg. per minute over the 60-minute period of observation.

contrast, glycine feeding was followed by a small rise in the blood sugar level in 3 of 4 subjects, the maximal change ranging from +14 per cent to +30 per cent, with a mean of +10 per cent change from the fasting level. It should be emphasized that attacks of clinical hypoglycemia invariably followed the ingestion of leucine in all 3 patients, whereas there were no clinical reactions following administration of glycine.

Adrenal steroids, when administered in quantities sufficient to raise fasting blood glucose values to normal levels, appeared to abolish leucine sensitivity. Under these conditions, leucine feeding resulted in maximal decreases in blood glucose concentration of only 12 and 3 per cent respectively, in Patients P.E. and G.M.

As seen in Figure 2, following the apparently complete surgical extirpation of tumor tissue from Patients P.E. and A.O., the administration of leucine did not provoke significant degrees of hypoglycemia; maximal changes in blood glucose concentration were +27 and −12 per cent, respectively.

Administration of leucine in functional hypoglycemia

It seemed pertinent to examine the effect of leucine in patients with functional hypoglycemia. In Table 4 is recorded the course of the blood glucose levels following ingestion of leucine by 2 normal subjects, 4 patients with functional hypoglycemia, and 1 patient who suffered from

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TABLE 4. EFFECT OF LEUCINE FEEDING ON THE BLOOD SUGAR LEVEL OF PATIENTS WITH FUNCTIONAL HYPOGLYCEMIA AND NORMAL SUBJECTS

Patient	Diagnosis	Blood glucose level (mg./100 ml.)												Maximal fall or rise	
		F#1*	F#2*	10 mins.	15 mins.	20 mins.	30 mins.	40 mins.	45 mins.	50 mins.	60 mins.	75 mins.	90 mins.	Absolute (mg./100 ml.)	Per cent of control
T.S., 41 W M	Normal	83	80	81		79		82			80			− 3	− 4
G.F., 30 W M	Normal	73	69	76		77		75			74			+ 4	+ 5
D.T., 40 W F	Functional hypoglycemia	68	68		71		70		68		71	70	68	0	0
M.G., 36 W F	Functional hypoglycemia	75		70		74	71	66		70	75			− 9	−12
A.M., 24 C F	Functional hypoglycemia	87	88	91		85	86	82		87	84	81	79	− 8	− 9
C.C., 72 W F	Functional hypoglycemia	lost					90		91		84	88	83	—	—
G.S., 34 W F	Functional hypoglycemia (prediabetes)	97			96		83		96		97	94	88	−11	−11

* F#1 and F#2 represent fasting blood sugar values obtained prior to the oral administration of leucine, 150 mg. per Kg.

hypoglycemic episodes secondary to the prediabetic state. These diagnoses were based on evaluations of the clinical status, oral and intravenous glucose tolerance curves, and intravenous tolbutamide responses. The detailed results of these studies will be reported in a separate communication (11). It is clearly evident from inspection of these data that no appreciable changes in blood glucose concentration occurred for periods up to ninety minutes after leucine feeding.

DISCUSSION

None of the 3 patients with pancreatic islet-cell tumor presented diagnostic difficulties at the time they were seen by the authors, although the findings in Patient A.O. had been equivocal during several previous careful studies. All had severe fasting hypoglycemia relieved by administration of glucose in the absence of hepatic, pituitary or adrenal insufficiency. Nevertheless, it is of interest that 1 of the 3 patients had a normal intravenous glucose tolerance curve and 2 patients failed to show insensitivity to intravenous glucagon-free insulin (10). Fajans and Conn (12) recently reported[2] that, in patients with organic hyperinsulinism, intravenous tolbutamide provokes a striking and prolonged exacerbation of hypoglycemia. We had the opportunity to evaluate this procedure in Patient A.O. and the results tend to confirm the original observation. To avoid inducing inordinate hypoglycemia, the sodium tolbutamide (1 Gm.) was injected intravenously only three hours after a meal. At this time the blood glucose level was 111 mg. per 100 ml. Subsequent blood glucose concentrations, determined on samples collected at fifteen-minute intervals during the first fifteen to sixty minutes of the test and at thirty-minute intervals thereafter up to one hundred and fifty minutes, were 82, 63, 96, 47, 35, 35 and 37 mg. per 100 ml. respectively.

It seems clear that, in the 3 patients studied, administration of leucine consistently and repeatedly provoked an accentuation of tumor-induced hypoglycemia.[3] Glycine feeding, on the other hand, had relatively negligible and inconstant effects on blood glucose levels. Taken out of the context in which they were conceived, the present studies could be interpreted

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[2] Since this article was submitted we have learned that a more detailed report by Fajans, Schneider, Schteingart and Conn appears on p. 371 of this issue of *The Journal of Clinical Endocrinology & Metabolism.*

[3] Fajans (13) has recently studied 2 patients with proven insulinomas under similar circumstances. In one there was a maximal fall, one hour after administration of leucine, of 12 mg. per 100 ml. (22 per cent) from a fasting level of 55 mg. per 100 ml. On another occasion in the same patient there was no change from the fasting level in a three-hour control period during which no amino acid was administered. The other patient showed no fall in blood glucose levels after either oral or intravenous leucine.

alternatively as indicating that ingestion of glycine, perhaps by a gluconeogenetic effect, counteracted the hypoglycemic effects of organic hyperinsulism whereas ingestion of leucine was ineffectual in this regard. We have no data to permit a decisive choice between these two possibilities, since blood glucose concentrations were not systematically followed when *no* amino acid was administered. However, the alternative hypothesis is less tenable in view of the patients' ability to tolerate fasting into the late morning hours under the conditions of these studies and the unequivocal documentation of sensitivity to leucine and insensitivity to glycine in some children with idiopathic hypoglycemia (2, 3, 4). In any case, broader experience with this phenomenon should clarify this aspect of the problem.

To our knowledge, leucine-induced accentuation of hypoglycemia has not been previously reported in patients with islet-cell tumor, although in one of the children studied by Cochrane *et al.* (2) islet-cell hyperplasia was found at operation. The occurrence of "leucine sensitivity" in children with idiopathic hypoglycemia and in adults with organic hyperinsulism suggests that a common mechanism may be operative in both disorders, but the nature of this relationship requires clarification. Several clinical differences are apparent. Hyperfunctioning islet-cell tumors are rare in infancy; either histologically normal islets or islet-cell hyperplasia are the rule (14). In the adult the reverse is true, tumors being common and islet-cell hyperplasia rare (8, 14). Hereditary transmission of infantile idiopathic hypoglycemia (14), including the leucine-sensitive variant (15), is well documented, whereas no such familial incidence has been mentioned in reviews (8, 16, 17) of adult patients with organic hyperinsulinism. Moreover, differences in plasma insulin-like activity (ILA) have also been reported. Using the rat diaphragm technique, an increase in the level of plasma ILA has been found in only 1 of 8 children (2, 16, 18, 19). In contrast, 7 of 11 adults with proven islet-cell tumor were found to have high concentrations of plasma ILA (20).

The mechanisms involved in the phenomenon of sensitivity to leucine remain obscure. Two possibilities seem reasonable; 1) that leucine stimulates release of insulin from the pancreatic islets, and 2) that leucine potentiates the peripheral action of insulin, either directly or by inhibiting the activity of an insulin antagonist. The first hypothesis is attractive in view of the parallel behavior of amino acids and glucose with respect to insulin. For example, insulin lowers the blood levels of both glucose and amino acids (21). Furthermore, insulin enhances the peripheral uptake of glucose and amino acids (22, 23). Reasoning by analogy it would seem plausible that just as high levels of blood sugar stimulate the secretion of insulin, an increase in the blood level of an amino acid such as leucine might also result in stimulation of the beta cells of the islets. Indeed, using a sensitive

and specific immuno-assay, Yalow and Berson (24) recently reported that plasma insulin levels increased significantly in 4 of 6 patients with leucine-sensitive idiopathic hypoglycemia following the feeding of leucine. Similar studies are in progress for patients with islet-cell tumor (25).

Despite the appeal of the notion that leucine acts by enhancing the production of insulin, many of the data contained in this report are compatible also with the concept that sensitivity to leucine occurs in the presence of greater-than-basal insulin activity, and that leucine in some way potentiates the action of insulin. It is evident that administration of leucine does not invariably serve as an adequate stimulus for the production of hypoglycemia. There is now convincing evidence that leucine feeding has

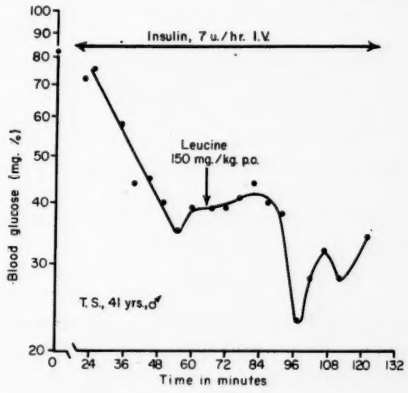

FIG. 3. Effect on blood sugar of the ingestion of leucine in a normal subject made hypoglycemic by the constant intravenous infusion of glucagon-free insulin.

no discernible influence on the blood sugar levels of euglycemic subjects. In our own experience this was true for Patients P.E. and A.O. after removal of the islet-cell tumor, for 2 normal subjects, and for 5 patients with functional hypoglycemia. These findings confirm those of Cochrane and associates (2). In addition, it appears (from admittedly too few observations) that sensitivity to leucine in patients with organic hyperinsulinism is greatly reduced or abolished when adrenal steroids are administered in sufficient dosage to establish an euglycemic state. These observations support the conclusion that there is no evidence for islet-cell stimulation by leucine during the postabsorptive state when endogenous beta-cell activity is presumably low. On the other hand, our findings, as well as those of the *in vitro* studies of Cochrane (3), suggest that leucine synergizes insulin activity. An experiment which may bear on this point is summarized in Figure 3. Shown in a semilogarithmic plot, is the apparent effect of leucine ingestion in a normal subject made hypoglycemic by the constant intra-

venous infusion of insulin. After the initial fall, the blood sugar level remained stable at approximately 40 mg. per 100 ml., but about twenty-five minutes after the ingestion of leucine there was a further precipitous drop to 23 mg. per 100 ml. Thus, both in magnitude and in time of occurrence, the results were similar to those observed in our patients with hyperinsulinism. Under the conditions of this experiment, however, endogenous insulin secretion is presumably suppressed, although the possibility remains that the administration of leucine provoked secretion of insulin.

These data are obviously inconclusive but favor the view that, in patients with islet-cell tumor, leucine acts by potentiating the peripheral action of insulin rather than by stimulating its secretion. However, there is no reason to believe that the proposed mechanisms are mutually exclusive.

Addendum

After this manuscript was submitted for publication, a report by Fajans *et al.* (26) appeared. These workers confirmed the observation that leucine does not affect the blood glucose level of normal persons. In addition, they were able to induce leucine sensitivity in subjects treated either with chlorpropamide or with ultralente insulin. Leucine-induced hypoglycemia was associated with an increase in plasma insulin-like activity. The interpretation of this latter finding remains uncertain, however, since the assay may not discriminate between an actual increase in plasma insulin concentration and a potentiation of insulin activity.

Acknowledgments

We wish to express our appreciation for the assistance provided by Dr. George Stuppy, Dr. Donald Tarun and Dr. Edwin Irons, whose patients served as the subjects of this report.

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ADRENOCORTICAL STEROID METABOLISM IN NEWBORN INFANTS. IV. PLASMA CONCENTRATIONS OF CORTISOL IN THE EARLY NEONATAL PERIOD*

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ABSTRACT

Determinations of material yielding the Porter-Silber reaction in extracts of umbilical cord plasma were compared with determinations of cortisol by isotope dilution. Cortisol values were also determined in pooled venous plasma from infants aged 3 to 96 hours. The presence of nonspecific background material and and possibly of other reacting steroid material in the cord plasma resulted in a distinct difference between values obtained by the colorimetric and by the isotope dilution techniques. The pooled plasma cortisol values rose in comparison with the cord blood values during the third to the sixteenth hours of life, and subsequently fell. Except in cord plasma, no circulating α-ketolic neutral steroids other than cortisol could be demonstrated by paper chromatography during the newborn period.

ALTHOUGH the unique anatomic structure of the adrenal cortex of the newborn human infant has led to speculation that the functional capacity of the infant gland may differ either quantitatively or qualitatively from that of the adult gland, reports of direct measurements of circulating adrenocortical hormone concentrations during this period are fragmentary. A relationship between maternal venous blood and cord blood concentrations of 17-hydroxycorticosteroids (17-OH-CS) has been demonstrated by several investigators (1–7) and estimation of the concentrations of these steroids during the first week of extra-uterine life has been reported by Klein and his co-workers (8, 9). These measurements of circulating 17-OH-CS have, in most cases, utilized the Nelson-Samuels modification of the Porter-Silber reaction (10) which measures cortisol and other adrenocortical steroids possessing the 17,21-dihydroxy-20-ketone side-chain. Since, with this method, hyperbilirubinemia and other factors (ACTH stimulation) which alter the nature of nonspecific "background" material may require unique interpretation of the results (11), the present

Received October 10, 1960.

* Supported in part by a grant from the USPHS (Grant A3107).

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study was undertaken to determine the specific content of cortisol in the plasma of normal full-term newborn infants.

The broad aims of the study were: 1) to measure by a specific isotope dilution method the concentration of circulating free cortisol in cord plasma and in pools of plasma obtained from infants during successive 12-hour periods during the first week of extra-uterine life; 2) to compare these values with those obtained using the Porter-Silber reaction; and 3) to determine whether there are present in cord plasma and in the plasma of newborn infants substances other than cortisol that contribute to or interfere with measurement of the steroid by the Nelson-Samuels method.

METHOD

Collection of samples

All blood was collected under sterile conditions using dry heparin as the anticoagulant. The blood was centrifuged immediately after collection and the separated plasma was frozen and stored at 0°C. until the time of assay. The pool of cord plasma was obtained from samples of the mixed arterial and venous blood of the umbilical cord. The amount obtained at each delivery was approximately 5 ml. of plasma. Four pools were collected—pools A and B during the summer and fall, pool C during the winter, and pool D during the spring. The postnatal plasma pools were obtained from blood samples collected from normal healthy full-term infants in the nursery. The internal jugular vein was used for venesection; the amount of plasma obtained from each infant was 2–3 ml. Each infant was subjected to only one venipuncture.

Extraction

At the time of assay, a known quantity of cortisol-4-C^{14} with a specific activity of 7785 counts per minute per microgram[1] was added to the pool. The maximal added quantity was .274 μg. per pooled sample. After thorough mixing, each sample was diluted with an equal volume of distilled water. Extraction with 600 ml. of methylene dichloride, previously purified by passage over silica-gel columns, was carried out at room temperature in a Rinco ball extracter (12). Extraction time, based on the size of the plasma sample, varied from eight to twenty-four hours. Extraction efficiency, as measured by the recovery of added radioactivity, varied between 61 and 81 per cent. Steroids more polar than cortisol were, therefore, inefficiently extracted. However, subsequent extraction of the plasma residues with ethyl acetate, followed by paper chromatography of the extracts, showed no blue tetrazolium-staining material polar to cortisol. After extraction, aliquots of the recovered methylene chloride were taken for estimation of Porter-Silber reacting material.

Measurement of Porter-Silber reacting material

a. An aliquot of the methylene-chloride extract of the original plasma pool was washed with weak alkali and water, passed through anhydrous sodium sulfate, and dried in an air stream. The dried residue was taken into chloroform and the 17-hydroxycorticoid content was determined by the method of Nelson and Samuels (10). Blank solu-

[1] Generously supplied by the Endocrine Study Section of the National Institutes of Health. Analysis showed 89 per cent purity on paper chromatograms, and correction for this is included in all calculations.

tions, and standard solutions at four appropriate concentrations were similarly chromatographed and determined with each batch.

b. A second aliquot of the original methylene-chloride extract was processed according to the method of Peterson *et al.* (11). This method differs from that of Nelson and Samuels in that no chromatographic columns are used and a series of blank solutions of varying combinations are analyzed and used to correct for interfering chromogens. In this study, spectrophotometric density determinations were performed at 370 and 450 mμ, as well as at 410 mμ.

Measurements of cortisol by in vitro *isotope dilution*

The remainder of the methylene chloride was washed three times with 0.01 *N* sodium hydroxide and three times with distilled water, filtered over anhydrous sodium sulfate, and dried in a film evaporator.

Peterson's method for corticosterone (13) was adapted to determine cortisol. The method provides a means of quantitation on the basis of the principle of isotope dilution. Cortisol-4-C^{14} of determined specific activity is added in a trace amount to the plasma pools before any processing is begun. Once mixed, therefore, this tracer will be recovered, lost, or destroyed in exact proportion to the cortisol natively present in the original plasma. Compared with the values obtained using other steroid procedures, these analytical results are relatively independent of incomplete extraction and other losses unavoidably incurred. The results are dependent upon final isolation of cortisol in high purity from paper chromatograms and the subsequent determination of its specific activity. The change in specific activity from that of the original cortisol-4-C^{14} and the amount of carrier cortisol originally added with the isotope are used to calculate the unknown content of cortisol in the original plasma sample. To modify the original method for the determination of cortisol, the area of the paper chromatogram containing cortisol and an area immediately adjacent (for blank determinations) were cut out and eluted. The cord-plasma cortisol areas were chromatographed a second time on the E_2B system of Eberlein (14) to ensure the isolation of cortisol from nonspecific background fluorescence. Counting of C^{14} was performed in a liquid scintillation counter using two fifths of the eluted material in a counting mixture of toluene with 0.3 per cent PPO. Counts were recorded for a time sufficient to obtain 3 per cent counting accuracy and corrected for background counts and for instrument variation, using a sealed standard. Data obtained with these modifications have agreed well with data using the Porter-Silber reaction (11) in the plasma of subjects older than those reported here (15).

Included in Table 5 is the ratio of the cortisol in the original sample, to the added carrier cortisol as C^{14} steroid. In all samples a ratio in excess of 3 to 1 was achieved, allowing for the greatest accuracy and validity of this procedure (16). In addition, it may be seen that even with the multiple tests which were performed on each extract of plasma, the size chosen for samples was sufficient to allow measurement of fluorescence on amounts greater than 1 microgram. The fluorometer setting for samples is such that fluorescent background material from the chromatograms is at a very low comparative level.

RESULTS

Porter-Silber reacting material

The data obtained using the modified Nelson-Samuels procedure for estimation of 17-OH-CS in the pooled plasma of newborn infants are summarized in Table 1. The consistently high absorption of light at 370

TABLE 1. CORD PLASMA 17-HYDROXYCORTICOSTEROID CONCENTRATION BY THE MODIFIED METHOD OF NELSON AND SAMUELS (10)

Sample	Plasma equivalents (ml.) of methylene chloride	Optical density			Calculated 17-OH-CS* (μg./100 ml.)
		370 mμ	410 mμ	450 mμ	
A	8	.483	.345	.213	0
B	8	.550	.410	.298	0
C	4	.310	.220	.148	0
D_1	5	.960	.800	.685	0
D_2	5	.990	.810	.680	0

* Using the Allen correction.

mμ following color development with acid phenylhydrazine resulted in values less than zero when the Allen correction was used for calculation (17). Such a result could be obtained either if the actual level of 17-OH-CS were low, or if the levels were low only in comparison with an interfering substance present at high concentrations in the plasma of newborns. This same aberrant spectral absorption pattern has been noted in the plasma of young infants. Table 2 represents a calculation of all data obtained in this laboratory using the methodology described during the time-period required to accumulate the data on the newborns presented here. It is included as a basis of reference for the findings in newborn infants. Comparisons of the ratio of densities at the 3 wave lengths indicated that the density readings at 370 mμ and 450 mμ were relatively higher in all chromatographed material including the standards themselves. Although a full explanation of this change of relationship is not forthcoming, several points demand attention. The data for determination of crystalline cortisol include many more samples of high steroid content than do the data for

TABLE 2. ATYPICAL SPECTRAL ABSORPTION OF PLASMA EXTRACTS IN NEONATAL PERIOD USING THE NELSON-SAMUELS METHOD (10)

(All density readings for each wave length were totaled. The sum of the 410 mμ readings was arbitrarily assigned the value of 1.000 and the sums of the readings at 370 mμ and 450 mμ are expressed as fractions of the sum of readings at 410 mμ.)

Sample	No. of determinations	Absorption spectra		
		370 mμ	410 mμ	450 mμ
Crystalline cortisol	33	.786	1.000	.478
Crystalline cortisol after elution from Florisil column	64	.857	1.000	.555
Plasma (individual samples) from infants 0–7 days	10	1.213	1.000	.728
Plasma (individual samples) from children 1–12 months	25	.949	1.000	.645
Plasma (individual samples) from children 1–15 years	120	.980	1.000	.621

the plasma samples. A level of standard was always included which was likely to be higher than any sample in the batch. By itself, this resulted in the inclusion of more high 410 mμ readings. The calculated 410 mμ density of the column standards is always lower than its equivalent unchromatographed crystalline cortisol density due to small losses of steroid and to inclusion of nonspecific background from the column (18). It is for these reasons that column standards and blank are always included. The ratio of calculated densities of chromatographed/unchromatographed standards during this period of study was 83.9 per cent ±1.5 (S.E.M.). The effect of these differences in recovery and background is such that the samples of higher steroid content (5 and 7 μg.) are relatively less affected than those of lower content (1 and 3 μg.), accounting at least in part, for the differences in ratios shown in Table 2. It is evident, however, that the mean ratio of the 370/410 mμ readings was above 1.0 only in the samples from very young infants, in marked contrast to the ratio in the samples from older infants and children.

Accordingly, an attempt was made to circumvent this problem by the use of a method which incorporates a plasma blank (plasma extract plus sulfuric acid-ethanol reagent) into the procedure (11). The data obtained by this method are summarized in Table 3. It appears from these data that the spectral absorption of the background material (blank) in no case follows a straight line in the region studied; therefore, the results obtained using these two modifications of the Porter-Silber reaction, uncorrected by plasma blanks, were considered unsuitable for accurate estimation of cortisol in plasma from human newborn infants. Examination of the "corrected" optical densities (Table 3) indicates that in 2 samples, material was still present that resulted in high optical density at 370 mμ, obscuring the appearance of peak absorption at 410 mμ. In no sample was there a linear character of the background with respect to wave length, to justify the use of Allen's formula for calculations (17). This problem was recognized as a potential source of error by Peterson *et al.* (11). Calculations by the recommended method (using the 410 mμ readings only) are also included in Table 3. It appears that the discrepancy between results obtained by the two methods of calculation increased as the ratio of sample to background decreased. The 17-hydroxycorticoid values calculated from these data, using the corrected optical densities, were within the very broad range of values reported in the literature (Table 4). Although no data on this point are presented in the papers cited here (1–7), Ely and Klein have found similar aberrant spectra in this age group (19, 20).

Cortisol concentrations as measured by in vitro *isotope dilution*

The calculated data obtained from the 4 cord plasma pools are summarized in Table 5. These values for cortisol concentration, ranging from

TABLE 3. CORD PLASMA 17-HYDROXYCORTICOSTEROID CONCENTRATION BY THE MODIFIED METHOD OF PETERSON (11)

Sample	Plasma equivalents (ml.) of methylene chloride	Optical density			Calculated 17-OH-CS (μg./100 ml.)	
		370 mμ	410 mμ	450 mμ	A*	B**
A	13	Sample 1.350	1.282	1.107		
		Blank .735	.575	.480		
		Corrected .615	.707	.627	20.6	66.3
B	13	Sample .602	.585	.428		
		Blank .240	.163	.125		
		Corrected .362	.422	.303	21.5	39.6
C	3.3	Sample .482	.408	.330		
		Blank .287	.194	.140		
		Corrected .196	.214	.192	18.9	79.1
D_1	5	Sample .187	.153	.087		
		Blank .024	.017	.013		
		Corrected .163	.136	.074	16.0	33.6
D_2	5	Sample .120	.117	.058		
		Blank .028	.038	.027		
		Corrected .092	.079	.031	16.0	19.5

* Calculated using the Allen correction on the corrected optical densities.
** Calculated by 410 mμ densities only.

7.8 to 9.4 μg. per 100 ml., were well below the mean values for individual samples reported by other authors using methods which detect all free 17-OH-CS in cord plasma. The data obtained on pooled samples of the peripheral venous plasma of newborn infants are also summarized in Table 5. A rise to twice the average concentration found in cord plasma occurred before or during the third to the twelfth hour of life; thereafter there was a fall in the concentration of cortisol to levels below those found in cord plasma.

Chromatographic studies

Cord plasma pools. A compound believed to be cortisone was detected in the cord plasma pools. Tentative identification was based on the identity of running rates between this compound and authentic cortisone in two solvent systems, blue-tetrazolium staining, production of fluorescence in ultraviolet light after spraying with 10 per cent sodium hydroxide, demonstration of peak absorption of light in methanol at 240 mμ, and a typical Porter-Silber color reaction. Small amounts of material more polar than cortisol were detected in the chromatograms of the larger cord plasma pools by staining with blue tetrazolium. These were not characterized

TABLE 4. COMPARISON OF DATA REPORTED IN THE LITERATURE

Authors	Method	Source	Free 17-OH-CS (μg./100 ml.)
Migeon, Keller, Holmstrom (26)	Nelson-Samuels	Cord blood; 19 normal vaginal deliveries	21.0
Elsner, Geyer, Kölbl (4)	Nelson-Samuels	Cord blood; 28 normal deliveries	31.0 (10.0– 54.6)
Klein, Fortunato, Papadatos (8)	Nelson-Samuels	Sera from cord blood; 23 normal deliveries	21.4
Kawahara (3)	Nelson-Samuels	Cord blood (mixed): 12 multiparous mothers 8 primiparous mothers 9 low-forceps delivery for fetal distress	 16.3 25.7 25.6
Migeon *et al.* (1)	Nelson-Samuels	Umbilical vein blood; 9 normal deliveries	23.2 (0.2– 43.5)
Gemzell (2)	Nelson-Samuels	Umbilical cord blood; 47 vaginal deliveries	22.80 ± 1.89
Bayliss *et al.* (5)	Nelson-Samuels (Bayliss-Steinbeck modification)	Umbilical cord blood (mixed) primiparous mothers (mean) 7 multiparous mothers	25 (12 – 50)
Seeman *et al.* (6)	Nelson-Samuels; 410 mμ reading only	25 infants; cord blood	55.06 (7.2–138.4)
Varangot *et al.* (7)	Nelson-Samuels (Bayliss-Steinbeck modification); 410 mμ reading only*	20 infants; cord blood	42.54 (12.0–112.8)
This study	Porter-Silber (Peterson modification)	Pooled cord blood; mixed 4 pools	18.6 (16.0– 21.5)
This study	Isotope dilution	Pooled cord blood; mixed 4 pools	8.55 (7.76– 9.42) cortisol only

* Sulfuric acid-ethanol blank for each sample.

further. Corticosterone was not identified. In addition, a material absorbing ultraviolet light and running between cortisone and corticosterone on the B5 system was studied. The average migration rate was 4.1 times that of cortisol. This material, probably nonsteroidal in nature, was apparent after color development with sulfuric acid-ethanol reagent, exhibited intense absorption of light at and below 370 mμ which rapidly diminished with increasing wave length, and may be one of the substances that accounted for the failure of the cord plasma samples to show maximal ab-

TABLE 5. CORTISOL CONCENTRATIONS IN PLASMA
(isotope dilution method)

Source of sample	Volume of sample (ml.)	Cortisol (μg./100 ml.)	Cortisol (μg./sample)	Ratio of cortisol in sample to added cortisol-4-C^{14}
Cord blood A	400	8.3	33.1	120.8
Cord blood B	400	7.8	31.0	113.3
Cord blood C	96	9.4	9.0	32.99
Cord blood D	500	8.7	43.7	106.9
3–12 hours	45	16.5	7.4	13.6
12–24 hours	42	10.2	4.1	7.8
24–36 hours	53	5.4	2.8	5.2
36–48 hours	25	7.5	1.9	3.4
48–60 hours	67	6.0	4.0	9.8
60–72 hours	65	7.0	4.6	8.3
72–84 hours	47	8.2	3.9	9.4
84–96 hours	38	11.3	4.3	7.8

sorbance at 410 mμ in the Porter-Silber color reaction. Its characteristics are not those of Seconal, Demerol or Hykinone—the drugs used in the mothers from whose infants these samples were obtained. Neither are the characteristics those of caffeine. The possibility that this may represent a metabolite of one of these drugs has not been excluded.

Blood plasma pools. No steroids other than cortisol were detected in the chromatograms of the pooled plasma from the blood of neonates. The quantities of plasma used were such that steroids less polar than cortisol and possessing a Δ^4-3-ketone configuration or an α-ketol side-chain in concentrations as low as 2 or 3 μg. per 100 ml. of plasma would probably have been detected in all samples, with the exception of the 36-hour to 48-hour pool.

DISCUSSION

The presence in methylene-chloride extracts of cord plasma of a substance or substances reacting with sulfuric acid-ethanol reagent to yield a pattern of spectral absorption beyond the wave lengths 370 mμ to 450 mμ, and which does not follow a straight line relationship, appears to make the results obtained by the Nelson-Samuels method subject to a different interpretation in the newborn infant than is usual in adult subjects. The introduction of a plasma blank into the method appeared, at times, to correct this spectral anomaly, but the persistently high uncorrected optical densities obtained at 370 mμ indicated that some material other than cortisol reacting with the acid-phenylhydrazine was present in concentrations high enough to interfere with the typical spectral absorption pattern of the 17-OH-corticosteroids. Use of the Allen calculation may be hazardous in this age group, therefore, even when a plasma blank is employed, unless it is shown that the background absorption is linear with respect to wave length.

The values obtained by the use of a method specific for cortisol were well below the mean of values for individual samples reported by other authors and below the values which were obtained in our laboratory on the same pools using the Porter-Silber reaction. Whether the difference was due to methodology, or whether it was also due in part to the presence of 17-OH-corticosteroids other than cortisol cannot be answered with the data at hand. The finding of a compound presumed to be cortisone on the chromatograms of the cord plasma pools would indicate that part of the difference was probably due to the presence of this compound. If so, there is no way of estimating the ratio of cortisol and cortisone by the colorimetric methods, and the results are the sum of the concentrations of each.

Migeon *et al.* (1) and Gemzell (2) reported that the ratio of maternal to fetal plasma free 17-OH-CS levels is between 5:1 and 2:1. Migeon *et al.* (1)

showed that material eluted from the cortisol area of a paper chromatogram accounted for only 69 per cent of the Porter-Silber chromogens of a chloroform extract of umbilical cord blood pooled from 3 vaginally delivered infants. If maternal plasma contained cortisol as its only free 17-hydroxycorticoid, the values reported here for cortisol in cord plasma would suggest that a higher maternal-fetal ratio exists than is indicated by their data. However, Cohen *et al.* (21) tentatively identified cortisone in plasma pools from women in the third trimester of pregnancy, so that estimation of cortisol alone in the plasma of the mother at parturition will be necessary to establish the true value of this ratio. Since cortisone in the fetus may also be partially transplacentally derived, specific methodology will be necessary to establish maternal-fetal ratios of cortisol and cortisone separately.

The rise in the concentration of hydrocortisone in the pooled plasma of newborn infants during the first 12 hours of extra-uterine life is of interest. Statistical comparison of the 3–12 hour value with all other values for cord and infant plasma through 84 hours indicates that a variation of this magnitude is highly unlikely, using a statistical test of confidence for single specimens on the basis of a series of replicate analyses (22). A few of the infants represented in this pool may have shown extremely high concentrations to account for the high pool value. Aside from such a sampling artifact, either mobilization from extravascular sites to the circulation, or increased production of cortisol by the infant's adrenals could account for this early postnatal rise. The routine care of each infant included in the study was interrupted only to obtain one blood sample in an attempt to prevent the possible effect of repeated venipuncture and blood loss on the state of adrenal activity. The excretion of 17-OH-CS in the urine on day 1 is often at a lower level than on subsequent days, making the redistribution theory attractive (23). Recently it has been suggested that the half-life of administered cortisol is markedly prolonged in the neonatal period (24). The data of Cranny *et al.* (25), although in apparent disagreement with the foregoing suggestion, clearly show that an increase of cortisol dosage from 1 to 2 mg. per kilogram of body weight results in a significant prolongation of the rate of disappearance of free 17-OH-CS from the blood. This extreme sensitivity to dosage level has not been observed in adults. Such effects as these could likewise account for an apparent rise in concentration in the face of a steady small adrenal output. A gradual decrease of plasma concentration is shown during the period of life from 12–36 hours (Fig. 1). Further redistribution out of the plasma of an unchanging amount of cortisol, suppression of adrenal steroid production, or an abrupt increase in degradation or excretion, could equally well explain the subsequent decline in concentration.

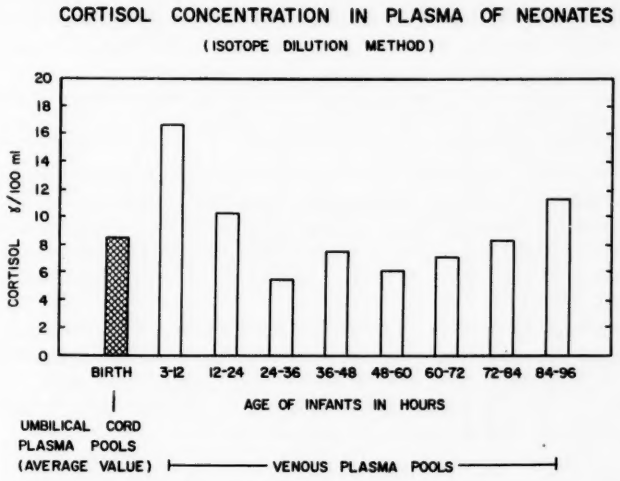

FIGURE 1

During the remainder of the period of study, the plasma cortisol values gradually increased with age, the 84–96 hour pool yielding the highest subsequent value (Fig. 1). No statistical significance should be attached to these increases, as reported here. Average values previously reported for blood 17-OH-CS during this period of life were 2 μg. per 100 ml., or lower (8). The described interference with the Porter-Silber reaction may account for this discrepancy. The gradual rise of plasma cortisol concentration demonstrated by our data is in keeping with a similar change of average 17-OH-CS excretion in the urine from the second to the sixth day of life (23). Considerable variation from infant to infant was noted in the urinary excretion values; this could also be true of plasma levels, but since the data reported here were obtained on pools of plasma, there was no knowledge of the individual variations within the pool. We were unable to identify any other steroidal material in chromatograms of the extracts of these plasma pools obtained from the blood of newborn infants. It appeared therefore that, as in the adult, cortisol is the major circulating free 17-hydroxycorticoid in normal newborn infants.

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BLOOD CORTICOTROPIN (ACTH) LEVELS IN CUSHING'S DISEASE*

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ABSTRACT

The present study was undertaken in search of a definitive answer to the question of whether the pituitary secretes abnormal quantities of corticotropin (ACTH) in Cushing's disease (hypercortisolism due to bilateral adrenal hyperfunction). It was postulated that if the primary disorder in Cushing's disease were a derangement of pituitary function it should not be corrected by removal of the adrenal glands. In such patients it should then be possible to control plasma cortisol levels while observing plasma ACTH levels. Two groups of patients were studied: 10 who had been treated for Cushing's disease by bilateral adrenalectomy, and 8 who had Addison's disease without prior history of Cushing's disease. Although the amounts of cortisol administered and the plasma 17-hydroxycorticosteroid values were similar for the two groups, blood ACTH levels were consistently higher in the patients with Cushing's disease. It is concluded that for any given level of plasma cortisol, the patient with Cushing's disease maintains a higher-than-normal level of blood ACTH.

INTRODUCTION

ALTHOUGH it has long been considered that excessive secretion of corticotropin (ACTH) might be the cause of Cushing's disease,[1] definitive proof in the form of a consistent demonstration of elevated blood ACTH levels has been lacking. Jailer *et al.* (1) reported that the plasma of patients with Cushing's disease, when administered to hypophysectomized rats, provided partial protection against adrenal atrophy. Adrenal ascorbic-acid depleting activity was not detected in the plasma of such patients; assays of steroidogenic activity were not performed. The precise nature of the plasma agent, therefore, remains in doubt.

Elevated plasma ACTH values have been reported in occasional cases of Cushing's disease (2), but most investigations have failed to demonstrate

Received September 24, 1960.

* Supported in part by grants-in-aid, (A-3166) and (2A-5092), from the U. S. Public Health Service.

[1] In this paper the term "Cushing's disease" will be used to designate *hypercortisolism due to bilateral adrenocortical hyperfunction*, in contrast to "Cushing's syndrome" which designates hypercortisolism of *any* etiology.

ACTH in the blood of patients with this disorder (3). Part of the problem has been methodological. Techniques for assaying ACTH have not been sensitive enough to measure the levels of this hormone in the blood of normal subjects. Therefore, the possibility that blood ACTH levels in Cushing's disease might be higher than normal but too low to quantitate, has not been adequately explored (3). In the present study, a method of concentrating plasma ACTH has been employed in combination with a sensitive bioassay technique, thus making it possible to obtain quantitative measurements of ACTH in concentrations as low as 1 milliunit per 100 ml. of plasma.

Further difficulty in assessing the role of ACTH in Cushing's disease has derived from the fact that the blood cortisol level is a crucial determinant of ACTH secretion. Cortisol restrains the secretion of ACTH, so that in general the higher the plasma cortisol level, the lower the rate of ACTH secretion. Patients with Cushing's disease characteristically have elevated levels of plasma cortisol, and it might be supposed that this high corticosteroid concentration exerts some restraint upon the secretion of ACTH in these patients. In previous studies of blood ACTH in Cushing's disease, this crucial variable has been uncontrolled. A fundamental tenet of the present study is that before one can judge whether a given level of blood ACTH is normal or abnormal, it is necessary to take into consideration the blood cortisol level. It is postulated that if in Cushing's disease the fundamental disorder be one of deranged secretion of ACTH, this disorder should not be corrected by removal of the adrenal glands. Following adrenalectomy, however, patients with Cushing's disease could be made comparable to a suitable control group with respect to blood cortisol levels. A suitable control group could be composed of patients with Addison's disease resulting either from primary adrenal disease or from bilateral adrenalectomy for reasons other than Cushing's disease. Both groups of patients, then, would lack adrenal function. Both would be maintained with normal plasma cortisol levels by exogenous means. The two groups would differ, however, in a fundamental respect: one would have had Cushing's disease; the other would not.

METHODS

Clinical material

Ten patients who had previously undergone bilateral adrenalectomy (subtotal in 2 cases) as treatment for Cushing's disease were studied. None exhibited clinical or radiologic evidence of a pituitary tumor. Eight patients with Addison's disease, 1 of whom had previously undergone bilateral total adrenalectomy for metastatic breast carcinoma, served as the "control" group. Corticosteroid replacement therapy for each patient is summarized in Table 1. Treatment in each case was constant for at least one week prior to the time blood was obtained for assay of ACTH.

TABLE 1. AMOUNTS OF CORTICOSTEROIDS ADMINISTERED, AND PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND ACTH

Values designated by ">" were not assayable with precision because the smallest amount of plasma extract used produced a maximal adrenal response. A second (incomplete) assay with plasma from Patient M.J. yielded an estimated mean of 80 milliunits per 100 ml. Values designated by "<" were not assayable with precision because the largest amount of plasma extract used produced a negligible adrenal response.

Patients	Dosage of steroid therapy		Plasma 17-OH-CS levels (μg./100 ml.)		Plasma ACTH levels (milliunits/100 ml.)		
	Cortisol	Fludrocortisone	Free	Conjugated	Estimate (mean)	95% confidence limits	Lambda
Cushing's Disease							
P.S.	10 mg./ 8 hrs.	0.1 mg./day	9	4	3	2– 5	.15
H.B.	10 mg./ 8 hrs.	0.1 mg./day	4	7	6	3–12	.38
M.J.	10 mg./ 8 hrs.	0.1 mg./day	20	7	>16 (est. 80)	—	—
C.W.	10 mg./ 8 hrs.	0	6	7	>10	—	—
P.D.	10 mg./ 8 hrs.	0.2 mg./2 days	11	6	26	15–44	.22
V.P.	10 mg./ 8 hrs.	0	4	—	7	4–13	.24
V.C.	10 mg./ 8 hrs.	0.1 mg./day	14	7	5	3–10	.25
F.E.	10 mg./ 8 hrs.	0.1 mg./day	8	10	14	9–24	.20
C.L.*	5 mg./ 4 hrs.	0	9	10	5	2–14	.45
R.J.*	0	0	(urinary 17-OH-CS twice normal; 15 mg./day)		8	4–16	.30
Average			9.4		>10.0		
Controls							
R.B.	10 mg./ 8 hrs.	0	15	8	<0.5	—	—
S.S.	10 mg./ 8 hrs.	0	16	—	<0.9	—	—
G.B.	10 mg./12 hrs.	0.25 mg./week	10	7	1.2	0.7–2.0	.19
E.K.	10 mg./ 8 hrs.	0.1 mg./day	8	7	1.3	0.7–2.3	.21
A.G.	10 mg./ 8 hrs.	0.1 mg./day	6	6	<0.5	—	—
J.F.	10 mg./ 8 hrs.	0.1 mg./day	3	6	<0.5	—	—
L.L.	10 mg./12 hrs.	0	—	—	0.5	0.4–0.7	.22
J.V.	0	0	—	—	1.1	0.5–2.3	.25
	(*dl*-aldosterone, 200 μg. 0/6 hrs., i.m.)						
Average			9.7		<0.8		

* Patients C.L. and R.J. had undergone subtotal adrenalectomy.

Cortisol and cortisol metabolites (17-hydroxycorticosteroids)

Plasma "free" 17,21-dihydroxy-20-keto-corticosteroids ("17-hydroxycorticosteroids"; 17-OH-CS) were determined by the Peterson modification (4) of the method of Silber and Porter. Plasma 17-hydroxycorticosteroid glucuronides were determined in similar fashion using extracts prepared by the method of Bongiovanni (5).

Collection of blood for ACTH assay

By gravity flow through polyethylene tubing, a sample of approximately 100 ml. of venous blood was collected directly into chilled, heparinized polypropylene flasks. Plasma was immediately separated by centrifugation at 4° C. A 10-ml. portion of the plasma was used for 17-OH-CS determinations and the remainder was used for ACTH assays. When possible, extraction of ACTH was begun immediately; otherwise the plasma was kept frozen until extraction could be initiated.

Extraction of ACTH

The carboxylic resin, Amberlite IRC-50 in the finely ground form XE-64, was processed according to the method of Hirs *et al.* (6), following which the resin was washed successively with 50 per cent acetic acid, pyridine acetate, 2 *N* hydrochloric acid, and 5 per cent acetic acid. Adsorption and elution of ACTH was accomplished by a modification of the method of Dixon (7). Two volumes of 50 per cent acetic acid (v/v) were

added to the plasma. Adjustment to pH 2 was accomplished using hydrochloric acid. The mixture was stirred while heating at 70–80° C. for thirty minutes. The mixture was then diluted with cold water so as to achieve a final acetic acid concentration of 5 per cent (v/v) and a pH of approximately 3. Resin, constituted as a slurry in 5 per cent acetic acid, was added to the plasma-acetic acid mixture so that 1 Gm. of the resin was added for each ml. of the original plasma. The resin-plasma-acetic acid mixture was stirred for four to eight hours. The resin was then drained and washed with approximately 1 liter of 1 per cent acetic acid. ACTH was eluted from the resin with 100 ml. of 52 per cent acetic acid (twice) followed by 100 ml. of pyridine acetate (30 ml. of pyridine plus 4 ml. of glacial acetic acid, diluted to 100 ml. with water). The eluates were dried under partial vacuum in a rotary evaporator at temperatures below 40° C. The final trace of pyridine acetate was evaporated under a stream of nitrogen. Just prior to assay, the dried eluates were reconstituted in aqueous solution containing 0.1 per cent albumin and 0.8 per cent sodium chloride adjusted to pH 2 with hydrochloric acid. Recovery of small amounts of ACTH (20 milliunits) added to a pool of human plasma was of the order of 70 per cent.

Assay of ACTH

Bioassays were performed employing the technique of Lipscomb and Nelson (8). Male Sprague-Dawley rats, each weighing 200–250 Gm., were hypophysectomized by the parapharyngeal approach three hours prior to the assay. Unknown plasma extracts, standard solutions of ACTH, or placebos were injected via the femoral vein over a one-minute period. One minute later, aqueous sodium heparin (250 U.S.P. units) was given by way of the femoral vein. Seven minutes after injection of the test solution, complete collection of the blood flow from the left adrenal vein was begun and continued for exactly three minutes. The blood was immediately centrifuged. Plasma corticosterone content was determined by the fluorometric technique of Silber *et al.* (9).

Standard solutions of ACTH were prepared by dissolving U.S.P. corticotropin reference standard in albumin-saline solution. Placebo injections consisted of albumin-saline.

Ratios of potency and 95 per cent confidence limits were calculated by the method of Sayers *et al.* (10). In each assay at least 2 dose levels of standard and 2 of unknown plasma extract were employed. Three or 4 rats were used at each dose level.

Preparation of equipment

Whenever possible, polypropylene containers and pipettes were used. All plasticware or glassware used in ACTH extraction or assay procedures was rendered free of residual ACTH contamination by soaking in hot trisodium phosphate and detergent solution for at least thirty minutes prior to washing. Only distilled deionized water was employed in the extraction and assay procedures.

RESULTS

Plasma 17-OH-CS

The plasma levels of cortisol and cortisol metabolites in patients with Addison's disease were similar to those in patients who had previously been adrenalectomized because of Cushing's disease (Table 1).

Plasma ACTH

Whereas plasma 17-OH-CS levels were similar for the two groups of pa-

tients, plasma ACTH levels were consistently higher in the patients who had previously been adrenalectomized for Cushing's disease. Individual values ranged from <0.5 to 1.3 milliunits per 100 ml. of plasma in patients with Addison's disease, and from 3 to 80 milliunits per 100 ml. in patients who had been adrenalectomized for Cushing's disease. Thus there was no overlap between these two groups (Fig. 1 and Table 1).

DISCUSSION

It appears that the excessive secretion of cortisol which characterizes Cushing's disease occurs as a result of excessive secretion of ACTH by the pituitary. Normally the ACTH-cortisol secreting system is self-regulating.

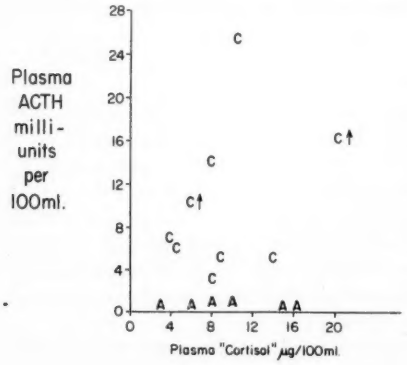

FIG. 1. Simultaneous plasma cortisol and plasma ACTH levels in patients with Addison's disease ("A") and those who had previously undergone bilateral adrenalectomy for Cushing's disease ("C"). Arrows indicate that ACTH activity exceeded the designated levels but could not be estimated with precision because the smallest doses employed produced maximal adrenal responses.

A rise in the blood cortisol level restrains secretion of ACTH, thereby curtailing further secretion of cortisol. In the patient with Cushing's disease, a similar regulatory system appears to operate at an abnormally high level; pathologically excessive amounts of cortisol are required to restrain the ACTH-secreting mechanism in such patients.

Indirect proof that adrenal secretion in Cushing's disease is ACTH-dependent has been derived previously from the demonstration that adrenal steroid output can be curtailed by large doses of ACTH-suppressing agents (11).

Presumably ACTH secretion is governed by the blood levels of nonprotein-bound cortisol, whereas the measurements in this study were concerned with total (protein-bound plus nonprotein-bound) cortisol. Available evidence indicates no characteristic deviation from normal in cortisol-binding protein levels in Cushing's disease (12). It may be assumed, therefore, that the results of the present study were not systematically influenced by increased protein-binding of cortisol among patients with Cushing's disease.

One cannot properly define a "normal" ACTH blood level without relat-

ing it to the level of cortisol. What might be a normal level of ACTH for a subject with a normal cortisol level would be inappropriately high for a subject with an elevated cortisol level. The present study represents the first deliberate effort to control blood cortisol levels while evaluating ACTH secretion in Cushing's disease.

At any given level of plasma cortisol, patients with Cushing's disease maintain a higher blood level of ACTH than do subjects without Cushing's disease. Thus when plasma cortisol levels are normal, the normal person has levels of ACTH which are high enough to maintain adrenal activity but not high enough to be readily measurable by direct assay. At comparable plasma cortisol levels, the patient with Cushing's disease has levels of blood ACTH which are so high that they can readily be measured (3 to 80 milliunits per 100 ml.). When plasma cortisol levels are raised to the range usually observed in untreated Cushing's syndrome, the normal person ceases to secrete ACTH, as indicated by the adrenal atrophy and unresponsiveness which ensue if such high plasma cortisol levels are artificially maintained for prolonged periods. With comparable levels of plasma cortisol, however, the patient with untreated Cushing's disease persists in secreting ACTH—enough, in fact, to stimulate his adrenal glands to excessive activity even though not enough to produce blood levels of ACTH which are readily measurable by direct assay. It is only with very great elevation of corticosteroid levels that the patient with Cushing's disease experiences complete suppression of ACTH secretion (11).

Excessive secretion of ACTH in Cushing's disease provides an explanation for all of the known features of the disorder. Adrenal hyperplasia, adrenal hypersecretion, and adrenal hyperresponsiveness to standard ACTH infusion (13, 14) are all known to occur when ACTH is administered over long periods to normal subjects. In order to explain the loss of diurnal variation in adrenal secretion which is commonly observed in Cushing's disease, it is only necessary to postulate a loss of diurnal variation of ACTH secretion—a possibility which is currently under investigation. The fact that Cushing's disease can be cured by hypophysectomy or by irradiation of the pituitary, and the fact that adrenal secretion can be curtailed by administration of sufficient quantities of exogenous corticosteroids are consistent with the view that Cushing's disease is due to excess ACTH. There is at present no evidence to support the hypothesis that Cushing's disease is an intrinsic adrenal disorder involving "primary" bilateral adrenal hyperplasia and hypersecretion.

Nelson *et al.* (15) reported high ACTH levels in the plasma of patients with pituitary tumors who had previously been adrenalectomized for Cushing's disease. Results of the present study indicate that a supra-normal blood level of ACTH following restoration of corticosteroid levels to normal

is characteristic of Cushing's disease in general and is not limited to patients with pituitary tumors.

The fact that pituitary secretion of ACTH is excessive in Cushing's disease appears now to be firmly established. The question of *why* the secretion of ACTH is excessive in Cushing's disease remains to be answered.

Acknowledgments

The authors gratefully acknowledge the valuable advice of Dr. George Sayers and Dr. Don H. Nelson concerning methods for assaying ACTH. Cortisol was generously provided in the form of "Cortef" by The Upjohn Company, Kalamazoo, Michigan.

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THE PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO CORTICOTROPIN, SU-4885 AND LIPOPOLYSACCHARIDE PYROGEN*†

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ABSTRACT

Two different mechanisms of pituitary activation were evaluated in 34 normal subjects, 5 patients with anterior pituitary insufficiency, 3 with adrenocortical insufficiency, 2 with Cushing's syndrome, and 3 with acromegaly, by studying serial changes in plasma 17-hydroxycorticosteroid levels in response to intravenously administered purified lipopolysaccharide pyrogen (Lipexal) and a propanone compound, SU-4885. These responses were compared to those observed following the intramuscular administration of 80 units of corticotropin gel. Similar studies were performed on 15 subjects maintained with corticotropin or corticosteroid therapy. The plasma 17-hydroxycorticosteroid responses to these tests provide a means for distinguishing between normal subjects and patients with panhypopituitarism, Addison's disease or Cushing's syndrome. Four hours after the fourth injection of corticotropin gel (administered intramuscularly in doses of 80 units every eight hours) there is complete separation between the ranges of response in these various groups of subjects. Since the responses to SU-4885 and to Lipexal differentiate normal subjects from patients with panhypopituitarism, they may be useful in investigating pituitary adrenal inhibition. Using these technics, pituitary-adrenal responsiveness following corticosteroid therapy was occasionally found to be suppressed, whereas following corticotropin therapy no suppression could be demonstrated. Evidence is presented which supports the concepts that 1) the site of suppression by corticosteroid therapy may be at the adrenocortical as well as at the pituitary level, and 2) there may be two mechanisms for activation of pituitary

Received September 9, 1960.

* Presented in part at the Meeting of the Southern Society for Clinical Research, January 22, 1960, New Orleans, Louisiana, and in part at the Forty-Second Meeting of The Endocrine Society, June 9–11, 1960, Miami Beach, Florida.

† This study was aided in part by grants 2A-5053, A-1265 and A-3555 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland; grant No. 342, American Medical Association; and funds from The Upjohn Company.

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corticotropin—one through negative feed-back stimuli (SU-4885), and one through stressful stimuli (Lipexal).

INTRODUCTION

ALTHOUGH numerous experiments have been designed to investigate the mechanisms concerned with adrenocortical responsiveness and with the release of adrenocorticotropin (ACTH) from the pituitary, the various factors involved have not been completely clarified. Since the development of technics for the chemical estimation of plasma corticoids with the 17,21-dihydroxy-20-ketone grouping (1, 2), precise and prompt measurements of the adrenocortical responsiveness to ACTH have become possible. After the original description (3) of the changes in plasma 17-hydroxycorticosteroid (17-OH-CS) levels which occur following the intravenous administration of ACTH, a standardized four-hour intravenous test utilizing the plasma 17-OH-CS response was evolved (4).

In the past, the lack of a quantitative, stressful or nonstressful stimulus to secretion of ACTH has limited investigation of the mechanisms of pituitary activation and inhibition. Following the demonstration that the toxic substance, amphenone B, suppressed production of cortisol through interference with hydroxylation of the steroid molecule (5), structural modifications of this compound were made which resulted in the formation of 2-methyl-1,2-bis (3-pyridyl)-1-propanone (or SU-4885), an essentially nontoxic analog (6). SU-4885 is relatively specific as an adrenal 11β-hydroxylase inhibitor at low dosage levels in the dog (7) and in man (8–12). Quantitative measurements of the pituitary response to various stressful stimuli and the development of a standardized test to measure this response have been the basis of several studies (13–26). An increase in the release of ACTH and in the concentration of plasma 17-OH-CS following the administration of polysaccharide pyrogens has been demonstrated in animals (16–19, 22, 25). Melby (21) showed recently that the plasma 17-OH-CS response in man to a purified lipopolysaccharide pyrogen (Lipexal) from *Salmonella abortis equi* may be a valuable quantitative index of pituitary release of ACTH.

Since Ingle's (27) demonstration that the long-term administration of adrenocortical extract results in atrophy of the adrenal cortex in animals, studies on the mechanisms of suppression of pituitary-adrenal function have been extensive (9–14, 17, 20, 21, 23–25, 28–36). The sites of this suppression have remained an enigma, and studies on the quantitative relationship between the degree of suppression and the amount of corticosteroid administered have yielded extremely variable results.

The present study was designed to evaluate further the usefulness of changes in free plasma 17-OH-CS levels following the intramuscular ad-

ministration of ACTH and the intravenous administration of Lipexal and SU-4885, as aids in clinical diagnosis and as indices of pituitary-adrenal activation in control subjects and in patients with panhypopituitarism, Addison's disease, Cushing's syndrome or acromegaly. These studies were supplemented by others designed to investigate: 1) the site, degree and duration of pituitary-adrenal suppression following corticotropin and corticosteroid therapy; 2) a possible difference between the mechanisms of pituitary ACTH activation resulting from SU-4885 and that resulting from infusion of pyrogen; and 3) the usefulness of two methods of assay of plasma 17-OH-CS concentration following administration of SU-4885.

MATERIALS AND METHODS

Subjects

Fifty-five hospitalized patients were studied on the medical wards of the University Hospital and Birmingham Veterans Administration Hospital. They were classified in the following seven groups:

1. Thirty-four control subjects (22 males and 12 females) without known pituitary or adrenal abnormality, ranging in age from 10 to 70 years with a mean age of 37 years. Nine were normal subjects, 9 had rheumatoid arthritis, 7 had anovulatory menstrual cycles, 1 had an old traumatic compression fracture of the eighth thoracic vertebra, and 1 had healed minimal tuberculosis. ACTH tests were carried out in 21 subjects, SU-4885 tests in 11, Lipexal tests in 22, and all three tests in 8.

2. Five patients with panhypotituitarism ranging in age from 41 to 72 years with a mean age of 62 years. Four were hypophysectomized males,[1] 2 of whom were maintained with 0.1 mg. of 9α-fluorohydrocortisone (9α-FF) orally five times daily; 1 was a female with Sheehan's syndrome. Four of these patients underwent ACTH, SU-4885 and Lipexal tests, and 1 had ACTH and Lipexal tests only.

3. Three patients (1 female aged 17 and 2 males aged 19 and 64) with proven idiopathic Addison's disease were maintained with 0.1 mg. of 9α-FF orally five times daily. Only ACTH tests were performed.

4. One female (aged 23) with Cushing's syndrome due to proven adrenal hyperplasia, and 1 male (aged 19) with Cushing's syndrome due to presumptive adrenal hyperplasia. ACTH, SU-4885 and Lipexal tests were carried out in the female, but only an ACTH test in the male.

5. One female (aged 43) and 2 males (aged 29 and 50) with acromegaly. ACTH, SU-4885 and Lipexal tests were performed in 1 of these patients, and only SU-4885 tests in the other 2.

6. Six males, ranging in age from 20 to 50 with a mean age of 36 years, and 1 female aged 36 years, who had been maintained with exogenous glucocorticoids for varying lengths of time (Table 5). ACTH, SU-4885 and Lipexal tests were performed in 4, Lipexal tests in 2, and only an ACTH test in 1.

7. One male aged 36 years who had been maintained with 20 to 40 units of ACTH gel administered intramuscularly daily for three years. ACTH, SU-4885 and Lipexal tests were performed.

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[1] Patient No. 35 was hypophysectomized for diabetic retinopathy and Patients No. 36. No. 37 and No. 38 for chromophobe adenoma.

Methods

ACTH test. The administration of 9α-FF resulted in a slightly decreased response to the four-hour ACTH test in 1 normal male subject (No. 6). In the initial test his plasma 17-OH-CS level increased from 16.2 to 40.5 μg. per 100 ml. Two weeks later the ACTH test was repeated on the second day of 9α-FF therapy (given orally in a dosage of 0.1 mg. five times daily). This time the plasma 17-OH-CS level increased from 25.2 to 38.1 μg. per 100 ml. Although the final level was similar in both instances, the four-hour increment in the last test was less than that in the control test.

The four-hour intramuscular ACTH test was performed according to the technic of the Veterans Administration Cooperative Endocrine Study, by administering 80 units of depo-ACTH gel[2] intramuscularly to all subjects at 8:00 a.m. Blood samples were obtained immediately before injection and four hours later (12:00 noon). In 3 of the control subjects and in all of the patients with panhypopituitarism, Addison's disease or Cushing's syndrome, and in other selected patients, ACTH was continued every eight hours for seventy-two hours (9 doses). Blood was drawn at 12:00 noon on the second and third days. In some of the patients, samples were also obtained at 5:00 p.m. on the day prior to testing (in order to evaluate diurnal changes) and at 8:00 a.m. on the day following the 72-hour ACTH test.

Consistency of response to ACTH (Table 1). The consistency of the plasma 17-OH-CS response to 80 units of depo-ACTH gel administered intramuscularly was demonstrated by the relatively consistent four-hour responses of 5 control subjects to 3 tests repeated at weekly intervals (Table 1).[3]

SU-4885 test. The intravenous SU-4885 test was performed according to the technic of Bissell *et al.* (10) by administering 1.0 Gm. of SU-4885[4] dissolved in 250 ml. of 0.9 per cent sodium chloride solution, intravenously over a period of two hours from 8:00 a.m. to 10:00 a.m. Blood samples were drawn before the test, and at two, four and six hours after starting the infusion. Control studies were made on 2 normal subjects in a similar manner, except saline alone was substituted for SU-4885. In these 2 instances, the plasma 17-OH-CS levels showed little response; the pre-injection levels were 12.7 and 16.1 μg., the two-hour levels were 8.6 and 11.7 μg., and the six-hour levels were 9.6 and 15.0 μg. per 100 ml. No toxicity was noted in any of the patients as a result of the infusions of SU-4885.

Lipexal test. The intravenous Lipexal test was performed according to the technic of Melby (21) by administering 0.5 μg. of Lipexal[5] intravenously at 8:00 a.m. and obtaining blood samples immediately before, and four hours after the injection. In most cases, blood samples were also drawn one and two hours after the injection. In the majority of the subjects the administration of Lipexal was followed in one to two hours by an elevation of temperature to 100°–102° F., which subsided in three to four hours. The febrile response was accompanied in some cases by chills, headaches, nausea and, rarely, vomiting. Despite the administration of 9α-FF, hypotension developed in a patient with

[2] Kindly supplied by Dr. Harold Upjohn and Dr. Hubert C. Peltier of The Upjohn Company, in the form of depo-ACTH gel, 80 U.S.P. units per ml., in 5-ml. vials.

[3] These are a small portion of a large number of such comparisons to be reported elsewhere by the Veterans Administration Cooperative Endocrine Study.

[4] Kindly supplied by Dr. C. H. Sullivan of Ciba Pharmaceutical Products, Inc., in the form of SU-4885 ditartrate injectable solution, 100 mg. per ml., in 10-ml. ampoules.

[5] Kindly supplied by Dr. Fred H. Schultz of the Wander Company, in the form of Lipexal, 0.5 μg. of *Salmonella* lipopolysaccharide per ml., in 2-ml. ampoules.

TABLE 1. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO ACTH IN TESTS REPEATED AT WEEKLY INTERVALS IN 5 CONTROL SUBJECTS*

Patient No.	Week	Plasma 17-OH-CS level (μg./100 ml.)		
		Day before ACTH	ACTH Day 1	
		5 p.m.	8 a.m.	12 noon
15	1	6.2	10.6	42.8
	2	6.3	8.5	35.4
	3	5.3	6.7	30.8
16	1	3.7	12.2	26.4
	2	8.3	13.9	35.1
	3	6.5	13.9	27.0
17	1	3.5	11.1	23.4
	2	5.6	10.2	26.4
	3	7.0	16.9	28.7
18	1	4.1	11.1	32.6
	2	8.0	24.8	38.7
	3	7.2	10.7	23.9
19	1	16.2	14.8	53.1
	2	15.5	17.1	46.2
	3	10.5	20.7	54.4

* See Table 2 for clinical data.

panhypopituitarism, and the test was terminated by the intravenous administration of 100 mg. of hydrocortisone hemisuccinate. There was no obvious correlation between the clinical response and changes in plasma 17-OH-CS levels.

Determination of 17-OH-CS. All blood samples were obtained by withdrawing 30 to 50 ml. of venous blood into a heparinized syringe. The blood was centrifuged within one hour; the plasma was separated and kept frozen until determination of the free 17-OH-CS content. A modification of the method of Peterson *et al.* (37) was used for this determination in each instance. Several of the plasma specimens were also analyzed for 17-OH-CS content, using the modification of Gold *et al.* (38). The reported results are averages of duplicate determinations and are expressed as micrograms per 100 milliliters.

RESULTS

Intramuscular ACTH tests (Table 2, Figs. 1 and 2)

In the 21 control subjects, the mean plasma 17-OH-CS level increased from 16.4 μg. at 8:00 a.m. to 44.2 μg. per 100 ml. four hours after the intramuscular injection of 80 units of depo-ACTH gel. The mean four-hour increase in plasma 17-OH-CS concentration was 27.8 μg. ($\pm$9.5 S.D.), with a range of 12.3 to 48.8 μg. per 100 ml. In 3 of these subjects, in whom ACTH was continued every eight hours for seventy-two hours, the plasma 17-OH-CS level gradually increased to a mean of 60 μg. per 100 ml.

In 5 patients with panhypopituitarism the 8:00 a.m. mean level of 17-OH-CS was 2 μg. per 100 ml., with a slight increase four hours after ACTH to 7 μg. per 100 ml., and a continued but diminished rise throughout the

TABLE 2. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO ACTH

Subject No.	Age & sex	Diagnosis	Daily maintenance therapy	Plasma 17-OH-CS level (μg./100 ml.) Day before ACTH (5 p.m.)	ACTH Day 1 8 a.m.	ACTH Day 1 noon	ACTH Day 2 (noon)	ACTH Day 3 (noon)	Day after ACTH (8 a.m.)
1	30 M	Normal			32.4	57.1			
2	23 M	Normal			13.4	42.1			
3	23 M	Normal			23.9	47.1			
4	23 M	Normal			17.3	48.6			
5	24 M	Normal			17.4	66.0			
6	30 M	Normal			16.2	40.5			
7	10 M	Normal			2.6	51.4			
8	41 F	Rheum. arth.			22.5	50.7			
9	58 F	Rheum. arth.			15.1	49.2			
10	24 M	Rheum. arth.		10.6	19.9	40.8			
11	27 M	Rheum. arth.		13.6	28.9	47.6	58.8	58.9	59.8
12	58 M	Rheum. arth.		8.4	14.1	33.8	51.6	57.2	59.8
13	51 M	Old CVA		25.6	21.2	41.3			
14	44 M	Old CVA		7.8	16.4	47.2			
15	40 M	Old CVA		6.2	10.6	42.8			
16	51 M	Old CVA		3.7	12.2	26.4			
17	56 M	Old CVA		3.5	11.1	23.4			
18	69 M	Old CVA		4.1	11.1	32.6			
19	70 M	Old CVA		16.2	14.8	53.1			
20	47 M	Old fracture T_8			13.2	49.5			
21	50 M	Inactive TB			10.0	37.3	43.5	63.4	
	Mean			*10.0*	*16.4*	*44.2*	*51.3*	*59.8*	*59.8*
	S.E.			*2.18*	*1.47*	*2.19*	*4.42*	*1.85*	*0.0*
35	63 M	Hypophysectomy	0.5 mg.FF*	0.5	2.8	1.8	19.8	23.6	22.1
36	72 M	Hypophysectomy	0.5 mg.FF*		2.3	2.5	10.6	18.0	21.3
37	67 M	Hypophysectomy		2.9	2.7	22.6	40.6	48.7	48.4
38	70 M	Hypophysectomy		0.9	2.4	6.9	24.0	27.0	31.5
39	41 F	Sheehan's syn.			0.0	0.0	18.8	24.0	29.9
	Mean			*1.4*	*2.0*	*6.8*	*22.8*	*28.3*	*30.6*
	S.E.			*0.69*	*0.51*	*4.12*	*4.96*	*5.31*	*4.88*
40	19 M	Addison's dis.	0.5 mg.FF*	8.0	4.2	1.9	5.2	10.9	
41	17 F	Addison's dis.	0.5 mg.FF*	0.0	1.2	1.3	0.0	1.0	0.4
42	64 M	Addison's dis.	0.5 mg.FF*		1.8	0.0	2.2	3.1	4.1
	Mean			*4.0*	*2.4*	*1.1*	*2.5*	*5.0*	*2.3*
43	23 F	Cushing's syn.			21.4	68.4	139.6	121.5	155.5
44	19 M	Cushing's syn.		25.4	24.0	77.6	123.8	139.3	
45	43 F	Acromegaly			9.7	44.2			

* 9α-Fluorohydrocortisone.

72-hour test. Although the initial level of 2.7 μg. per 100 ml. in Patient No. 37 was lower than in any normal subject, the four-hour increase was 19.9 μg. per 100 ml., which was greater than in 3 of the 21 normal subjects. This suggests the possibility that hypophysectomy was not complete in this patient. In 3 patients with Addison's disease, there was a similar low mean level of 2 μg. per 100 ml. at 8:00 a.m., and virtually no increase throughout the 72-hour test (Fig. 1). In 2 patients with Cushing's syndrome and bilateral adrenocortical hyperplasia the plasma 17-OH-CS response was greater than normal four hours after ACTH, rising from 21.4 and 24.0 μg. to 68.4 and 77.6 μg. per 100 ml. respectively, and there was a markedly exaggerated rise throughout the 72-hour period to levels of 139.5 and 155.5 μg. per 100 ml. at the time of the last sample (Fig. 2). No overlap was observed in the ranges of the responses in these groups of patients. There was

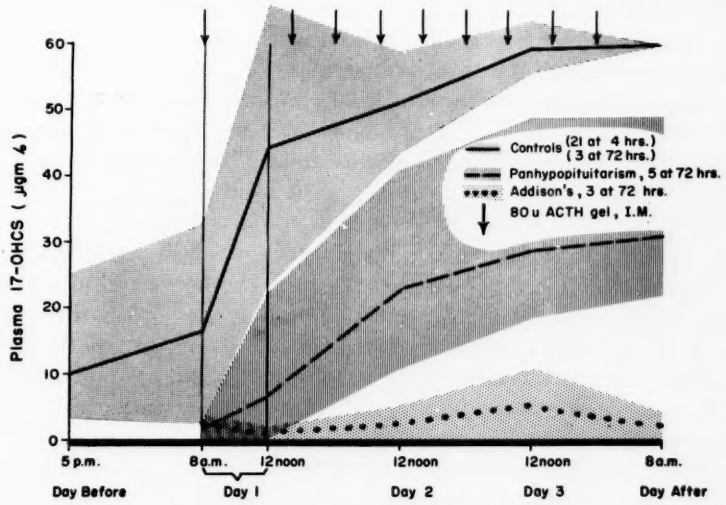

FIG. 1. Mean and range of the plasma 17-hydroxycorticosteroid response to ACTH in control subjects, patients with panhypopituitarism and patients with Addison's disease.

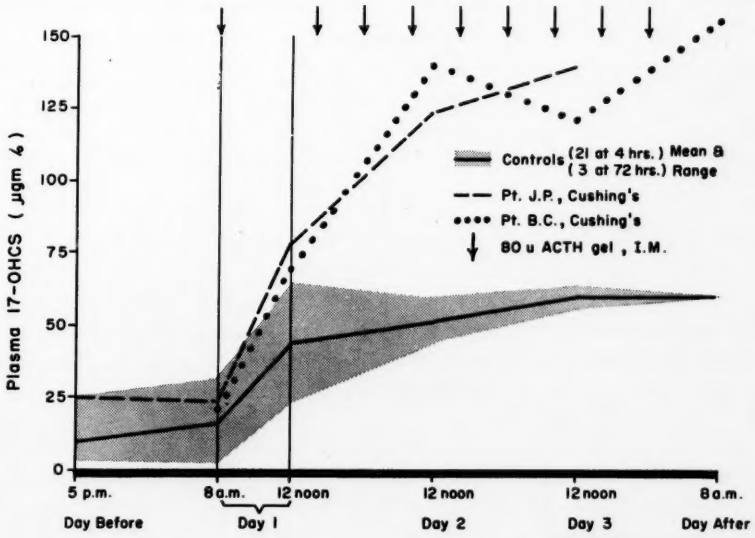

FIG. 2. Plasma 17-hydroxycorticosteroid response to ACTH in 2 patients with Cushing's syndrome. The mean and range of the control response are also shown.

complete separation of the range of plasma 17-OH-CS response at four hours, and the separation was unequivocal by twenty-eight hours. One patient with acromegaly exhibited a normal response.

The expected diurnal decrease at 5:00 p.m. was observed in 8 of the 10 control subjects tested. The 1 patient with Cushing's syndrome tested showed no diurnal decrease in the plasma 17-OH-CS level (Table 2).

Intravenous SU-4885 tests (Table 3, Fig. 3)

In 11 control subjects the mean initial 8:00 a.m. plasma 17-OH-CS level of 14.3 μg. per 100 ml. decreased to 7.1 μg. at two hours and increased to 13.9 μg. at six hours. The mean increment from the second to the sixth hour was 6.2 μg. with a range of 0 to 20 μg. per 100 ml. In 4 patients with panhypopituitarism the mean 8:00 a.m. level was 4.0 μg. and there was virtually no change at two, four or six hours. In 1 patient with Cushing's syndrome the 8:00 a.m. level of 14.4 μg. per 100 ml. decreased to 11.1 μg. at two hours and increased to 21.5 μg. at six hours—an increment greater than the mean increment observed in normal subjects (Fig. 3). In 3 patients with acromegaly the plasma 17-OH-CS responses were within the normal range (Table 3). The marked variability in the responses of the control subjects made interpretation of the results of this test difficult.

TABLE 3. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO SU-4885

Subject		Diagnosis	Daily maintenance therapy	Plasma 17-OH-CS level (μg./100 ml.)							
				Peterson method				Gold method			
No.	Age & Sex			Control	2 hrs.	4 hrs.	6 hrs.	Control	2 hrs.	4 hrs.	6 hrs.
1	30 M	Normal		8.7	2.4	4.2		9.4	10.6	10.9	7.3
2	23 M	Normal		9.1	5.5	12.0	13.3	10.7	5.8	13.6	16.2
3	23 M	Normal		8.5	10.6	7.3	12.0	12.3	10.6	15.4	14.5
4	23 M	Normal		11.3	6.4	11.8	12.7	12.8	14.6	15.3	10.6
5	24 M	Normal		13.2	4.2	6.1	24.2	7.0	5.3	11.9	12.7
22	30 M	Normal		14.0	3.0	9.6	8.5				
23	38 M	Normal		13.2	3.8	9.1	12.3				
10	24 M	Rheum. arth.		16.5	5.6	3.9	11.3				
11	27 M	Rheum. arth.		29.4	13.2		16.3				
12	58 M	Rheum. arth.		17.5	7.7		7.4				
20	47 M	Old fracture T_8		16.0	15.3	8.0	21.2	14.9	9.6	9.8	10.9
	Mean			*14.3*	*7.1*	*8.0*	*13.9*	*11.2*	*9.4*	*12.8*	*12.0*
	S.E.			*1.78*	*1.26*	*0.98*	*1.67*	*1.13*	*1.41*	*0.95*	*1.29*
35	63 M	Hypophysectomy	0.5 mg.FF*	3.4	2.6	2.4	4.0				
37	67 M	Hypophysectomy		4.6	1.7		2.1				
38	70 M	Hypophysectomy		1.8	0.9	1.0					
39	41 F	Sheehan's syn.						6.2	2.2	2.4	0.0
	*Mean***			*4.0*	*1.8*	*1.9*	*2.0*				
	S.E.			*0.94*	*0.34*	*0.48*	*0.11*				
43	23 F	Cushing's syn.		14.4	11.1	21.9	21.5		14.6	28.9	23.2
45	43 F	Acromegaly		17.6	18.9	12.1	7.4	12.6	7.8	15.0	13.1
46	29 M	Acromegaly		17.5	6.9	13.7	15.7				
47	50 M	Acromegaly		10.7	3.8	17.0	17.5				

* 9α-Fluorohydrocortisone.
** Includes results of test on Patient No. 39.

Comparison of two methods of assay of the plasma 17-OH-CS response to SU-4885

Nine patients received SU-4885 as previously described, and their plasma samples were analyzed for 17-OH-CS content by two methods (37, 38). The results are shown in Table 3 and Figure 4. Using the modification of Gold *et al.* (38), the mean plasma 17-OH-CS level at four hours was 15 μg. per 100 ml. as compared with 10 μg. per 100 ml. by the method of Peterson *et al.* (37). Although the difference in these values was not statistically sig-

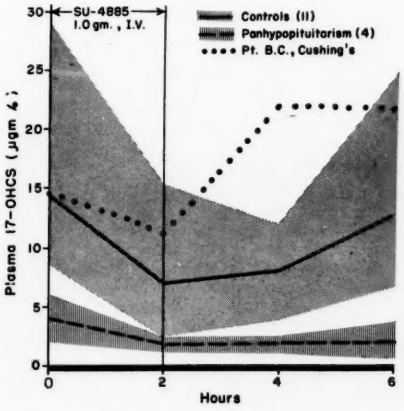

FIG. 3. Mean and range of the plasma 17-hydroxycorticosteroid response to SU-4885 in control subjects, patients with panhypopituitarism and 1 patient with Cushing's syndrome.

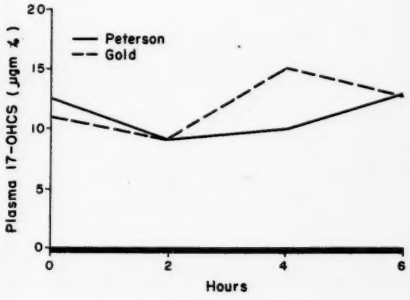

FIG. 4. Mean plasma 17-hydroxycorticosteroid response to SU-4885 in 9 patients —determined by both the Peterson *et al.* (37) and Gold *et al.* (38) methods.

nificant ($P > .05$), it did suggest the possibility that recovery of 11-deoxycortisol was greater by the Gold method. Using both methods, recovery experiments were performed on samples made by adding known amounts of 11-deoxycortisol to specimens of pooled plasma. The results confirmed the studies of Gold and his co-workers (38), who showed marked enhancement of 11-deoxycortisol recovery by his modification.

Intravenous Lipexal tests (Table 4, Fig. 5)

In 22 control subjects the mean initial 8:00 a.m. plasma 17-OH-CS level of 14.4 μg. per 100 ml. increased to 31.1 μg. per 100 ml. four hours following administration of Lipexal (Fig. 5). A progressive increase in the mean plasma 17-OH-CS level was seen at one and at two hours, when determined (Table 4). In these subjects the mean four-hour increase was 16.7 μg.

TABLE 4. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO LIPEXAL

Subject		Diagnosis	Daily maintenance therapy	Plasma 17-OH-CS level (μg./100 ml.)			
No.	Age & sex			Control	1 hr.	2 hrs.	4 hrs.
1	30 M	Normal		21.7			27.1
2	23 M	Normal		13.8			32.5
3	23 M	Normal		17.8			22.5
4	23 M	Normal		12.7			38.8
5	24 M	Normal		18.1			38.6
22	30 M	Normal		7.9	10.1	22.9	30.3
8	41 F	Rheum. arth.		13.8	18.3	23.0	14.8
10	24 M	Rheum. arth.		16.6	17.0	26.5	28.0
11	27 M	Rheum. arth.		19.6	27.6	39.5	29.2
12	58 M	Rheum. arth.		14.2	26.3	29.9	23.2
24	56 F	Rheum. arth.		21.5	16.9	21.0	33.1
25	16 F	Rheum. arth.		12.5		39.6	49.6
26	58 F	Rheum. arth.		12.0	15.3	22.5	19.0
27	40 M	Rheum. arth.		11.6	16.9	12.0	34.4
20	47 M	Old fracture T_8		14.6			33.8
28	29 F	Anovulatory		11.4			28.2
29	19 F	Anovulatory		7.0			31.5
30	21 F	Anovulatory		9.0			31.9
31	28 F	Anovulatory		16.4			31.9
32	23 F	Anovulatory		6.2			27.9
33	21 F	Anovulatory		11.3			26.6
34	24 F	Anovulatory		26.0			50.2
	Mean			*14.4*	*18.6*	*26.3*	*31.1*
	S.E.			*1.04*	*2.03*	*2.92*	*1.74*
35	63 M	Hypophysectomy	0.5 mg.FF*	2.2	2.6		
36	72 M	Hypophysectomy	0.5 mg.FF*	0.9	0.9	1.0	0.4
37	67 M	Hypophysectomy		4.6	6.9	7.4	
38	70 M	Hypophysectomy		1.0	1.2	4.7	7.3
39	41 F	Sheehan's syn.		3.0	2.0	0.0	0.0
	Mean			*2.3*	*2.7*	*3.2*	*2.6*
	S.E.			*0.69*	*1.08*	*1.71*	*2.37*
43	23 F	Cushing's syn.		21.4	15.2	18.5	24.4
45	43 F	Acromegaly		12.9			55.3

* 9α-Fluorohydrocortisone.

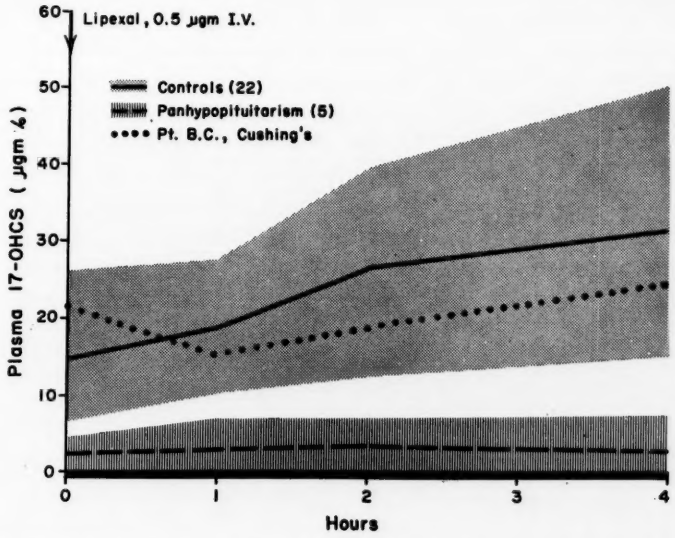

FIG. 5. Mean and range of the plasma 17-hydroxycorticosteroid response to Lipexal in control subjects, patients with panhypopituitarism and 1 patient with Cushing's syndrome.

($\pm$7.8 S.D.), with a range of 1.0 to 37.1 μg. per 100 ml. In 4 of the 9 subjects tested, the response was greater at two hours than at four hours. In 5 patients with panhypopituitarism (2 receiving 0.5 mg. of 9α-FF daily, and 3 untreated), the mean 8:00 a.m. plasma 17-OH-CS level was 2.3 μg. per 100 ml., and there was no significant change at one, two or four hours. There was no overlap in the ranges for these groups. In 1 patient with Cushing's syndrome the initial 8:00 a.m. level of 21.4 μg. per 100 ml. showed little change following administration of Lipexal (Table 4, Fig. 5).

17-OH-CS responses during long-term therapy with corticosteroids or ACTH (Table 5)

In an attempt to investigate the site and degree of pituitary-adrenocortical suppression following corticosteroid therapy, the plasma 17-OH-CS response to ACTH was measured in 7 patients who had been maintained with corticosteroids from three months to five years. In addition, 4 of them (A.A., C.T., E.H. and J.G.) underwent both SU-4885 and Lipexal tests, and 2 others (J.C. and D.H.) underwent Lipexal tests. Only 3 patients (A.A., J.C. and C.T.) had subnormal plasma 17-OH-CS responses to ACTH, and 2 of them (A.A. and C.T.) also had low or low-normal responses to SU-4885 and Lipexal, as would be expected. One patient (J.G.), who had a normal response to ACTH, showed decreased responses to

TABLE 5. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO ACTH, SU-4885, AND LIPEXAL IN PATIENTS RECEIVING LONG-TERM CORTICOSTEROID THERAPY

Subject		Diagnosis	Previous daily therapy	Duration of period without therapy	Plasma 17-OH-CS level (μg./100 ml.)													
					ACTH test						SU-4885 test				Lipexal test			
Name	Age & sex				Day before test (5 p.m.)	Day 1 8 a.m.	Day 1 noon	Day 2 (noon)	Day 3 (noon)	Day after test (8 a.m.)	Control	2 hrs.	4 hrs.	6 hrs.	Control	1 hr.	2 hrs.	4 hrs.
A.A.	50 M	Bronchial asthma	Dexamethasone, 0.75–2.25 mg., for 1 yr.	2–4 days	4.5	8.7	17.4				15.2	3.5	0.0	4.5	9.6	14.5	15.5	15.9
				2 wks.		12.2	15.0				5.3	0.1	8.3	5.9	8.1	7.4	11.1	16.3
				6 wks.		6.8	27.2				14.9	5.1	3.6	6.1	12.8	7.2	23.9	24.1
				10 wks.							18.5	6.3	9.4	15.0				
E.H.	27 M	Rheum. arth.	Cortisone, 50–75 mg., for 1 yr.	1 wk.		19.7	37.6				10.4	5.0	1.8	5.9	15.1	25.8	19.8	31.8
J.C.	32 M	Rheum. arth.	Prednisone, 15–30 mg., for 1 yr.	Prednisone, 15 mg. daily, continued		20.9	27.2	45.1	54.0	59.5					12.4	18.8	21.6	24.6
C.T.	36 F	Eczematoid dermatitis	Various steroid ointments for 1 yr.	Ointments continued	11.1	11.5	19.6	31.8			13.0	5.5		6.9	14.7	12.5	16.7	17.1
D.H.	67 M	Rheum. arth.	Dexamethasone, 0.75–1.5 mg., for 3 mos.	2–4 days	9.2	30.4	46.1	56.9	57.6	57.8					23.4	28.5	35.4	31.0
B.J.	20 M	Hypogonadism	Cortisone, 37.5 mg., for 6 mos.	0.5 mg. FF* daily	1.6	2.8	23.5	35.4	38.8	50.8								
J.G.	24 M	Sarcoidosis	Various steroids for 5 yrs.	2–4 days		3.1	43.5				13.9	7.6	6.6	7.1	6.7			8.9
								Gold method→			7.6	1.7	13.4	8.3				
H.Y.	36 M	Rheum. arth.	ACTH-gel, 20–40 units i.m., for 3 yrs.	2–4 days	1.7	13.2	37.4	65.1	58.0	56.4	17.4	8.7	15.0	25.3	25.3	24.1	39.2	46.0

* 9α-Fluorohydrocortisone.

SU-4885 and Lipexal, which would imply pituitary suppression. Another patient (E.H.), who had normal responses to ACTH and Lipexal, had a subnormal response to SU-4885, which suggests different mechanisms of pituitary activation. Surprisingly, 1 patient (J.C.) who had a subnormsl response to ACTH, had a normal response to Lipexal.

As seen in Table 5, there was no definite correlation between the degree of suppression and either the duration or daily dose of corticosteroids.

In 1 patient (H.Y.) who had been maintained with 20 to 40 units of ACTH gel intramuscularly daily for three years, there was a normal or high-normal response to all three tests (Table 5).

17-OH-CS responses during short-term corticosteroid or ACTH therapy (Table 6)

The plasma 17-OH-CS response to administration of Lipexal was measured in 7 control subjects following two to four days of exogenous corticosteroid therapy consisting of 100 to 200 mg. of cortisone intramuscularly daily. The studies were repeated following two to four days of exogenous ACTH therapy consisting of 80 to 240 units of ACTH gel intramuscularly daily in fractional doses.

The mean increases in the level of plasma 17-OH-CS four hours after administration of Lipexal in these 7 subjects were 11.9 μg. per 100 ml. before cortisone therapy and 7.1 μg. per 100 ml. after cortisone therapy. The difference between these means was not statistically significant ($P = .2$). However, it is of interest that the response after cortisone in 3 of the 7 subjects would be considered inadequate when compared to the previous response in our 22 control subjects. In addition, approximately half of the subjects showed their maximal response at two hours rather than four hours after administration of Lipexal. Evaluations based on the maximal response in all the subjects revealed increases in the mean plasma 17-OH-CS level of 16.0 μg. per 100 ml. before cortisone therapy, and 9.5 μg. per 100 ml. after cortisone therapy. The difference between these means was statistically significant ($P = < .05$).

The response to Lipexal after administration of ACTH was difficult to interpret because of the high initial plasma 17-OH-CS levels in 4 of the subjects. However, in the 3 (Nos. 10, 11 and 22) with initial levels within the normal range, the four-hour increases were 22.4, 9.6 and 11.4 μg. per 100 ml. before ACTH therapy, and 16.3, 11.7 and 3.1 μg. per 100 ml. after ACTH therapy, respectively. The maximal increases in these 3 patients were 22.4, 19.9 and 11.4 μg. per 100 ml. before, and 16.3, 11.7 and 19.9 μg. per 100 ml. after ACTH, respectively. Although, quantitatively, the suppression after ACTH therapy appeared to be less than after cortisone

TABLE 6. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO LIPEXAL AFTER SHORT-TERM CORTICOSTEROID OR ACTH THERAPY

Subject No.	Test	Plasma 17-OH-CS level (μg./100 ml.)			
		Control	1 hr.	2 hrs.	4 hrs.
22	Initial	7.9	10.1	22.9	30.3
	After ACTH*	14.5	7.8	17.1	30.8
	After Cortisone**	10.9	11.8	20.3	25.9
8	Initial	13.8	18.3	23.0	14.8
	After ACTH	41.8	39.1		70.2
	After Cortisone	19.1		26.6	27.4
26	Initial	12.0	15.3	22.5	19.0
	After ACTH	41.4	30.4	36.5	19.0
	After Cortisone	16.9	18.0	25.0	28.0
11	Initial	19.6	27.6	39.5	29.2
	After ACTH	11.3	11.3		23.0
	After Cortisone	22.0	23.5	22.4	18.0
10	Initial	16.6	17.0	26.5	28.0
	After ACTH	11.2	11.8	31.1	14.3
	After Cortisone	10.2	14.1	21.5	14.9
12	Initial	14.2	26.3	29.9	23.2
	After ACTH	34.0	28.2	22.8	20.4
	After Cortisone	16.8	15.7	22.7	24.7
27	Initial	11.6	16.9	12.0	34.4
	After ACTH	45.0	32.5	32.4	40.4
	After Cortisone	15.2	16.6	27.7	18.7
Mean	*Initial*	*13.7*	*18.8*	*25.2*	*25.5*
	After ACTH†	*28.4*	*23.0*	*27.9*	*31.2*
	After Cortisone	*15.9*	*16.6*	*23.7*	*22.5*

* 80–240 units of depo-ACTH gel intramuscularly for 2–4 days.
** 100–300 mg. of cortisone intramuscularly for 2–4 days.
† Interpretation limited due to high initial values.

therapy, the small number of patients did not permit drawing definite conclusions.

Recovery of pituitary-adrenal responsiveness following inhibition by corticosteroids (Table 5, Fig. 6)

Serial ACTH, Lipexal and SU-4885 tests were carried out in Patient A.A. following discontinuance of long-term corticosteroid therapy. The plasma 17-OH-CS responses to ACTH and Lipexal were depressed at one and at two weeks, but were normal at six weeks after discontinuing steroids. In contrast, the response to SU-4885 appeared to be less than normal at one, two and six weeks, but had returned to normal by ten weeks. The results in this one patient must be viewed in the light of the previously demonstrated variability of pituitary-adrenal responsiveness following inhibition by corticosteroids (11, 23, 29–31, 33).

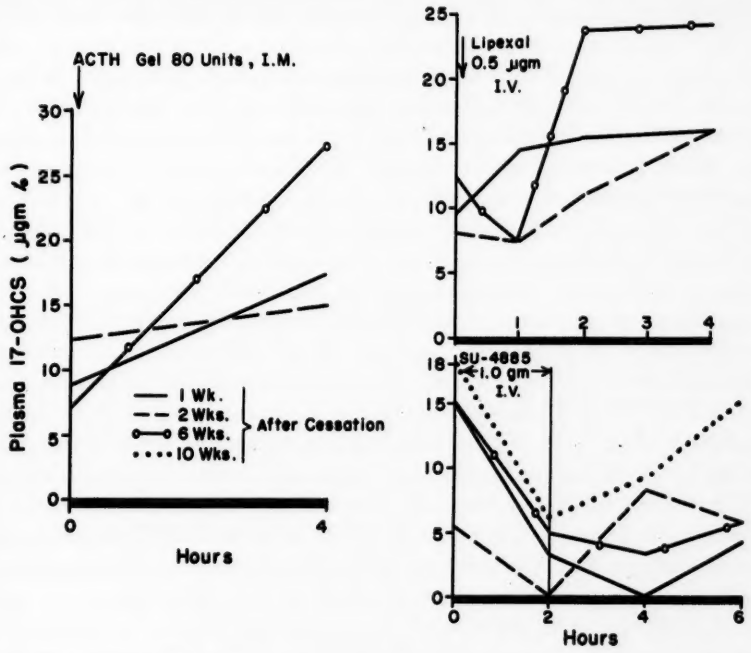

FIG. 6. Recovery of pituitary-adrenal responsiveness in Patient A.A. following discontinuance of long-term corticosteroid therapy.

Comparison of plasma 17-OH-CS responses to ACTH and Lipexal following ACTH and cortisone therapy in the same subject (Fig. 7)

An attempt was made to determine the site of inhibition of the action of exogenous steroids by comparing, in the same subject (No. 22), the plasma

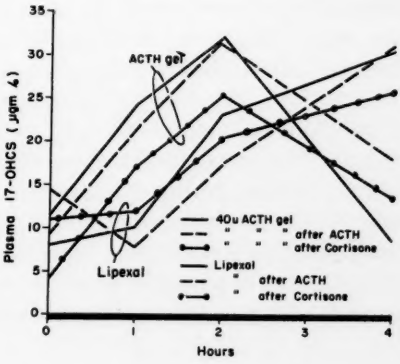

FIG. 7. A comparison in one control subject of the plasma 17-hydroxycorticosteroid response to ACTH and to Lipexal: 1) before and after two days of treatment with cortisone intramuscularly in a dosage of 100 mg. every eight hours, and 2) before and after two days of treatment with ACTH gel intramuscularly in a dosage of 80 units every eight hours.

17-OH-CS responses to ACTH and Lipexal before and after two days of therapy with cortisone intramuscularly in a dosage of 100 mg. every eight hours. Similar studies were made in the same subject before and after two days of therapy with ACTH gel intramuscularly in a dosage of 80 units every eight hours. Forty units of ACTH gel was selected as the testing dose in an effort to reproduce the plasma 17-OH-CS increase obtained with 0.5 μg. of Lipexal. During each test, blood was drawn at zero, one, two and four hours. The maximal response to 40 units of ACTH gel intramuscularly in each of the three tests occurred at two hours, whereas the maximal response to Lipexal in each of the three tests occurred at four hours. The peak levels were identical in all instances except after cortisone therapy, when the peak levels (in both the ACTH and Lipexal tests) were depressed 5 to 6 μg. per 100 ml. Although these slight differences in the plasma 17-OH-CS response in one patient are not conclusive, the data are compatible with the hypothesis that the possible inhibition demonstrated was at the adrenocortical level rather than at the pituitary level, and that ACTH, in contrast to cortisone, did not inhibit either the adrenocortical or the pituitary response. Similar results have been obtained in animal studies demonstrating a direct inhibitory effect of corticoids on steroidogenesis by interference with the action of ACTH at the adrenal level (39).

DISCUSSION

The results of the present studies demonstrate the diagnostic and investigative usefulness of the plasma 17-OH-CS response to intramuscularly administered ACTH. The administration of 80 units of depo-ACTH gel was shown to produce a consistent and reproducible four-hour mean increase of 27.8 ($\pm$9.5 S.D.) μg. per 100 ml. in the concentration of plasma 17-OH-CS in control subjects. On continued administration of ACTH to control subjects there was a progressive but more gradual increase in the plasma 17-OH-CS level over a 72-hour period. Patients with panhypopituitarism showed a low four-hour mean increment in plasma 17-OH-CS of 5 μg. per 100 ml. following administration of ACTH. There was a progressive but markedly diminished increment in these levels throughout the 72-hour test. The rise in plasma 17-OH-CS concentration in 1 of the hypophysectomized patients (No. 37) was closer to that seen in normal subjects, which cast some doubt on the completeness of hypophysectomy in this patient. Similar studies in patients with Addison's disease demonstrated essentially no change in the low plasma 17-OH-CS levels. In 2 patients with Cushing's syndrome, plasma 17-OH-CS levels were markedly increased during a similar program of ACTH administration. Although the majority of investigators have also demonstrated an increased plasma 17-

OH-CS response in patients with Cushing's syndrome (3, 4, 33, 40–43), one group did not demonstrate a consistent increase in these patients (44). The lack of the diurnal decrease in the plasma 17-OH-CS concentration in our patient with Cushing's syndrome was in contrast to the decrease usually found in the control subjects, and emphasizes the possible usefulness of this finding in the diagnosis of Cushing's syndrome, as has been previously noted (45). Although there was complete separation of the range of plasma 17-OH-CS response at four hours in the studies reported here, the separation of the ranges for the various groups of subjects was unequivocal by twenty-eight hours. The lack of overlap in the ranges of the responses indicates the value of this procedure in the clinical diagnosis of these disorders.

The usefulness of the plasma 17-OH-CS response to intravenously administered ACTH in evaluating adrenocortical function has been demonstrated by several investigators (3, 4, 11, 23, 29, 31, 33, 40, 42–44), and that of the response to intramuscularly administered ACTH, by others (41, 44, 46–49). On assessment of the present studies and those of others, the critical importance of the type of ACTH, the dose, the route of administration, and the time of blood sampling, is apparent. The convenience of intramuscular injections and the separation of the various groups of patients by the method of ACTH administration employed in the present studies emphasize the usefulness of this technic in measuring adrenocortical function. Large-scale studies comparing the various responses to differing doses and routes of administration of ACTH are being made by the Veterans Administration Endocrine Cooperative Study.

The usefulness of the plasma 17-OH-CS response to intravenously administered SU-4885 as an index of pituitary responsiveness was evaluated in several types of patients. Although there was a relatively consistent pattern of response in normal subjects, the individual variation was so marked that quantitative standardization of the normal response was extremely difficult. At the end of the infusion there was a clear-cut decrease in the plasma 17-OH-CS level in most of the control subjects. This was followed by an increase at four and at six hours to a level which approximated that found in the control specimens. By using a plasma 17-OH-CS method (38) which is known to recover 11-deoxycortisol more efficiently than the one routinely employed (37), the mean increase at four hours was somewhat more evident. In patients with panhypopituitarism there was essentially no change from the initially low plasma 17-OH-CS levels, whereas in 1 patient with Cushing's syndrome there was a slightly exaggerated normal response to intravenously administered SU-4885. This latter finding has been noted previously (9, 10, 12) and might imply nor-

mal pituitary responsiveness. In 3 patients with acromegaly, the response could not be distinguished from that in normal subjects.

Although there was no overlap between the responsiveness of normal subjects and that of patients with panhypopituitarism in the present studies, the variability of the changes in plasma 17-OH-CS following administration of SU-4885 by the technic employed limits the usefulness of this test. High circulating levels of SU-4885 may completely inhibit steroidogenesis, whereas low levels inhibit only 11β-hydroxylase activity (7). Because of this dose-related suppression of steroidogenesis, precise control of the amount and the route of administration and the consequent circulating blood levels would be critical factors in standardizing any test using this compound. In addition, an assay method for plasma 17-OH-CS which is known to recover 11-deoxycortisol more efficiently should be used (Table 3, Fig. 4).

In the SU-4885 test, the stimulation of pituitary ACTH results from a decrease in the "negative feed-back mechanism." In contrast, the administration of the lipopolysaccharide pyrogen, Lipexal, provides a standardized and reproducible test of the pituitary's ability to respond to stressful stimuli. The present studies confirm and extend previous work which demonstrated the diagnostic usefulness of the plasma 17-OH-CS response to intravenously administered Lipexal (21). The present data indicate that the intravenous administration of 0.5 μg. of Lipexal produced a four-hour mean increase in the plasma 17-OH-CS level of 16.7 ($\pm$7.8 S.D.) μg. per 100 ml. in control subjects. In patients with panhypopituitarism there was virtually no change from the initially low levels, and in 1 patient with Cushing's syndrome there was no change from the initially high-normal level, following administration of Lipexal. There appeared to be no correlation between the febrile response and plasma 17-OH-CS response in any of the subjects.

Evidence has been obtained in the past which suggests that the response to fever and pyrogenic substances in some respects resembles the response to administration of ACTH but that urinary corticosteroid excretion may not be significantly altered (15, 26). In rats, a bacterial polysaccharide complex was shown to stimulate release of pituitary ACTH, and this response was blocked by pretreatment with cortisone (16, 17). The latter finding might explain the failure of the presently reported patient with Cushing's syndrome to respond to Lipexal. Such an explanation assumes an increased production of corticoids in this patient despite normal levels of plasma free 17-OH-CS at the time of the test. This assumption is supported by the markedly increased urinary excretion of total 17-OH-CS and the lack of a diurnal decrease in the level of free plasma 17-OH-CS in this patient. Further studies have confirmed and extended the stimu-

lating effect of pyrogenic materials on pituitary release of ACTH (18, 19, 22, 24, 25, 50).

The only standardized procedure other than the Lipexal test for evaluating the pituitary's response to stressful stimuli in man has been the effect of insulin administration and consequent hypoglycemia on the blood sugar and plasma 17-OH-CS levels (24).

Previous studies in both animals and man have clearly demonstrated decreased responsiveness of the pituitary adrenocortical system following administration of corticosteroids (9–14, 17, 20, 21, 23–25, 27–36). Prolonged administration of corticosteroids has been shown to deplete the pituitary ACTH content in the rat and in the dog (20, 36, 51, 52). In the only similar study carried out in man, a reduction in pituitary ACTH concentration and histologic changes were demonstrated in 1 of 4 patients who had received long-term corticosteroid therapy—this patient having been treated the most intensively (11).

Numerous clinical studies suggesting relative adrenal insufficiency associated with surgery or other stressful situations in patients during corticosteroid therapy have been reviewed (23). Although there is general agreement that this type of adrenocortical insufficiency does occur, there has been no consistent correlation between the degree of suppression as measured by ACTH responsiveness and either the total dose or the duration of corticosteroid therapy (11, 23, 29–31, 33). In an attempt to delineate the site of the possible suppression by exogenous corticosteroids, several investigators haves studied the pituitary-adrenal response to SU-4885 (10, 11, 12, 35). Although in most studies some degree of suppression has been noted, the interpretation of the results is difficult because usually the adrenocortical response to ACTH was not measured and the technic of testing was variable. Further evidence of decreased pituitary-adrenal responsiveness following exogenous corticosteroids has been demonstrated with another technic of pituitary stimulation using Lipexal (21). Our observations on the plasma 17-OH-CS response to Lipexal and ACTH in Patient No. 22 before and after administration of cortisone are compatible with the hypothesis derived from previous studies in man and animals that corticoids have a direct inhibitory effect on the adrenal cortex (35, 39).

The data reported here on several patients receiving both long-term and short-term corticosteroid therapy indicate that the plasma 17-OH-CS response to ACTH, and consequently to SU-4885 and Lipexal, may be suppressed in some but not in all subjects receiving corticosteroids. No consistent correlation was evident between the degree of suppression and either the dosage or the duration of corticosteroid therapy.

Study of the restoration of these different responses in a patient who had received long-term corticosteroid therapy indicates that the "nega-

tive feed-back mechanism," as tested by SU-4885, takes longer to be restored to normal than does the mechanism for the response to stress, as tested by Lipexal. This would support the suggestion that there is more than one mechanism for activation of pituitary ACTH (50).

In contrast to the suppression of pituitary-adrenal responsiveness following exogenous corticosteroids, it is of interest that previous studies have shown no clear-cut suppression following exogenous ACTH. Indeed, one group of investigators found an increased ACTH content of the pituitary following prolonged administration of ACTH in rats (34, 52). although another study demonstrated a decreased ACTH content under similar circumstances (53). There is some evidence that ACTH administered simultaneously with corticosteroids may preserve normal pituitary-adrenal responsiveness despite clinical symptoms of adrenocortical insufficiency (24). Other reports indicate that patients receiving long-term ACTH therapy may suffer from vascular collapse during surgery, but no quantitative adrenocortical hormone assays are reported (54, 55). More recent studies indicate that patients treated with exogenous ACTH prior to operation show a normal plasma 17-OH-CS response to surgery as well as to ACTH administered preoperatively (23). The present studies on a patient treated with ACTH for three years demonstrate that such therapy did not have the suppressive effect, either on pituitary or adrenocortical responsiveness, previously shown with corticosteroids. This lack of suppression by ACTH was also noted in another patient (No. 22) who was tested with ACTH and Lipexal before and after cortisone therapy and before and after ACTH therapy. A confirmation of these observations by applying similar technics to other patients receiving long-term ACTH therapy would be of great practical importance.

Acknowledgments

The authors wish to express their appreciation for the technical assistance given by Mrs. Barbara Shannon, Miss Mattie Gautney, Dr. Hugh Dempsey, Mr. John Morrow, Mr. Larry Skelton, Mr. Willard Starnes, Miss Mary Sheffield, Mrs. Hilda Harris, Miss Joyce Faught, Mr. Harry Germagian and Mr. Ray Lookofsky.

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RESIN UPTAKE OF I^{131}-TRIIODOTHYRONINE AS A TEST OF THYROID FUNCTION*

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ABSTRACT

A simple, reproducible, *in vitro* test of thyroid function was developed based upon the uptake of I^{131}-triiodothyronine from serum by the anion exchange resin, IRA-400, in the formate cycle. One milliliter of serum which had been equilibrated with I^{131}-triiodothyronine was added to 1 milliliter of resin in a calibrated tube. The mixture was shaken for ninety minutes and assayed in a well counter. After three washings with water the resin was counted again to determine the resin uptake. The findings (mean $\pm$ standard deviation) were: 1) hypothyroidism, 27 ± 3.3 per cent; 2) euthyroid subjects, 35 ± 2.2 per cent; 3) thyrotoxicosis, 53 ± 7.8 per cent. The elevated resin uptake values in active thyrotoxicosis fell after therapy. In pregnancy, the values were depressed to the hypothyroid level or lower. Contamination with organic and inorganic iodine did not interfere with the test.

THE present communication describes a simple, reproducible test of thyroid function in which the radioisotope tracer is added to the patient's serum *in vitro*. This technique offers distinct advantages. Valid results can be obtained in patients who have received organic or inorganic iodine, both of which interfere with conventional thyroidal I^{131} uptake and serum protein-bound iodine determinations. Moreover, in the test described, it is unnecessary to administer isotope to the patient.

Previous reports (1–3) have presented *in vitro* methods which possess these advantages. However, when the procedures for the erythrocyte uptake of I^{131}-triiodothyronine described by Hamolsky, Freedberg and coworkers (1, 2) and the resin uptake of I^{131}-thyroxine described by Mitchell (3) were repeated in our laboratory, we found limitations in convenience, reproducibility and diagnostic discrimination.

In the course of the present work a number of resins were tested for their ability to bind I^{131}-labeled thyroxine and/or triiodothyronine and their analogues. The following resins were studied and found less satis-

Received September 21, 1960.

* This work was carried out under contract AT (30-1)-2361 with the United States Atomic Energy Commission, and supported by grants from the American Cancer Society and the National Institutes of Health.

factory than the system finally selected: IRA-400 in acetate, chloride, iodide and hydroxyl cycles; IR-120; Dowex 50 W-X2; Dowex 1-X2 in acetate and formate cycles; and Dowex 2-X4 in acetate and formate cycles.

The analogues tested, in addition to L-thyroxine and triiodothyronine, included D-thyroxine and triiodothyroacetic acid.

In the method selected, the anion exchange resin, IRA-400, was used in the formate form. One milliliter of serum which had been equilibrated with I^{131}-labeled triiodothyronine was added to 1 milliliter of resin in a calibrated tube. The mixture was shaken mechanically for ninety minutes. Total radioactivity was assayed in a well-type scintillation counter. After three washings with water, the resin was counted again to determine the percentage of radioactivity bound, or "resin uptake."

MATERIAL AND METHODS

Preparation of the resin

IRA-400, an Amberlite resin, was obtained in the chloride form from the manufacturer (Rohm and Haas, Philadelphia, Pa.). The original material was converted to the formate form by washing with 1*M* sodium formate solution previously adjusted to pH 5.0 ± 0.2. The resin was washed in a large chromatography tube containing a coarse fritted disc. About 1,500 ml. of formate solution was required to convert 50 Gm. of resin completely to the formate form. The rate of efflux from the column did not exceed 150 to 200 ml. per hour, and the washing was carried out for at least ten hours. The flow was stopped overnight, with the resin remaining in contact with the formate solution. The next day washing was resumed. The washing was terminated when the effluent was free of chloride ion, as determined with dilute silver nitrate solution. The washed resin was stored at 5° C. under an equal volume of sodium formate solution (pH 5.0). Washed batches exhibited no changes in I^{131}-triiodothyronine uptake from sera after four months of storage.

Radioactive tracer

I^{131}-labeled L-triiodothyronine was obtained from Abbott Laboratories, and stored at 5° C. Addition of Red Cross human serum albumin to the tracer to a final concentration of 2.5 Gm. per cent served to stabilize the material beyond the manufacturer's stated expiration date, as judged by chromatography as well as by resin uptake results. The presence of albumin made no difference in the resin uptake, since identical results were obtained when fresh tracer without albumin was used. With added albumin, stability for eight to twelve weeks was not unusual. Appreciable breakdown to iodide, which occurred after prolonged storage, resulted in spuriously high resin uptake values, since iodide is strongly bound by the resin. The behavior of the tracer was checked by a known standard from a serum pool, run with each set of determinations.

Prior to use, the tracer was diluted 1:100 with water to a final concentration of 0.2 μg. per ml. or less. Diluted solutions were usually used up within a week or two; in a few instances stability for three to four weeks was demonstrated. Greater dilutions, such as 1:500 or 1:1,000, yielded identical results, and were sometimes used instead of 1:100. Triiodothyronine concentrations greater than 0.12 to 0.2 μg. per ml., such as attained in 1:100 dilutions, were avoided since excessive amounts yielded higher uptake results, as did addition of "cold" triiodothyronine.

Blood sera

Frozen sera were stable indefinitely. Repeated thawing and freezing had no detectable effect. Refrigerated liquid sera were not as a rule kept in this state more than a day or two.

A large pool of sera from the Medical Chemistry Laboratory discards was kept, and run each day as a standard to check the overall procedure (resin, tracer). Results were constant within ± 1–2 per cent.

Procedure

All operations until the final washings and count were carried out at 2–6° C. Test tube racks were placed in trays to which cold water and ice cubes were added. To a series of tubes each containing 0.2 ml. of the diluted tracer solution was added 2 ml. of each serum. The tubes were shaken thoroughly and left standing in the ice tray for fifteen to forty-five minutes while resin tubes were made ready.

The screw-cap counting tubes had been calibrated by etching at 1 ml. and 5 ml. Into each of a series of these counting tubes in the ice bath, the resin was delivered using a tube with an opening 7 mm. in diameter. With practice it was found possible to deliver the resin quite accurately to the 1 ml. mark of the screw-cap tube. Excess resin could be removed by suction with a capillary glass tube or a long blunted needle, but this was rarely necessary. The formate solution was removed by washing twice with approximately 5-ml. portions of water squirted with a wash bottle as a forceful jet on the tube walls above the resin. From each 2.2 ml. of serum-triiodothyronine solution, 1 ml. was added to duplicate resin tubes. The serum-resin mixtures were shaken briskly for ninety minutes in the cold room. Too violent shaking, which spattered the resin on the walls of the tubes, resulted in less consistent results. One satisfactory mechanical device was the Burrell wrist-action shaker, set at a slow speed.

At the conclusion of the shaking, the tubes were kept in an ice bath except for the one-minute period required for radioactive assay in a well-type scintillation counter. Warming to room temperature was avoided, since this was found to increase the resin uptake. After the determinations of total radioactivity, water washing was carried out with approximately 5-ml. portions squirted into the tubes with a wash bottle to stir up the resin. Water was added prior to aspiration of the serum already present, and this was followed by three additional water washings. In aspirating the washings, care was taken to avoid losing any particles of resin. After the first aspiration, the ice bath was no longer needed. After the final radioactive assay, the activity bound by the resin was determined as the percentage of the original radioactivity, and expressed as "resin uptake" values.

Duplicate determinations on a given serum usually agreed within 1–2 per cent, as did the values for a given serum run at different times.

The use of an ice bath (2–6° C.) afforded better reproducibility and discrimination between diagnostic categories than similar systems run at 27° C., 37° C., or "room temperature" with its unavoidable fluctuations.

CLINICAL MATERIAL

Normal control group

Blood samples were drawn from 35 non-fasting healthy employees, after physical examinations in the Personnel Clinic of the New York State Psychiatric Institute.

Thyrotoxicosis

This group consisted of 44 patients with active thyrotoxicosis verified by determinations of the thyroidal I^{131} uptake and the levels of plasma protein-bound iodine (PBI) or butanol-extractable iodine (BEI). In most instances there had been no previous treatment. Seven cases represented recurrence after thyroidectomy or cessation of antithyroid medication, or inadequate drug therapy.

Myxedema and hypothyroidism

In this group were 28 clinically typical cases of myxedema or hypothyroidism of varying degree, verified by determinations of the plasma PBI or BEI concentration, and usually by the thyroidal uptake of I^{131} as well. Four of these patients had hypopituitarism, 5 had hypothyroidism after I^{131} or surgical therapy, and the rest had spontaneous idiopathic myxedema or hypothyroidism. The patients either had not received previous treatment, or had discontinued it two months prior to study, except for 3 who had been receiving inadequate replacement therapy.

Pregnancy

Eleven pregnant women were drawn at random from the obstetrical clinic of the Columbia-Presbyterian Medical Center.

Estrogen-treated carcinoma patients

These 4 subjects had received 5 mg. or more of diethylstilbestrol daily for longer than a week. Three were men with prostatic carcinoma and 1 was a woman with mammary carcinoma.

Euthyroid subjects with marked abnormalities of serum proteins

Twenty-three sera demonstrating the most abnormal electrophoretic patterns available were obtained from the Medical Chemistry Laboratory of the Presbyterian Hospital. In all instances the serum albumin concentrations were markedly diminished, and the various globulin fractions were elevated, depending upon the diagnostic category. Clinical impressions of the 23 cases were as follows: acute and chronic liver diseases, 4; nephrotic syndrome, 2; gastro-intestinal disorders, 4; sarcoidosis, 2; cardiovascular and pulmonary diseases, 5; and miscellaneous, 6.

RESULTS

Under the conditions described, I^{131}-triiodothyronine and I^{131}-thyroxine were taken up completely from a saline solution; 86 per cent and 75 per cent respectively were taken up from a 5 Gm. per cent solution of human serum albumin.

The resin uptake values in thyroid disease differed from those in the normal controls, as illustrated in Figure 1. The deviations from normal in the groups with thyrotoxicosis, myxedema, or pregnancy were highly significant statistically ($P < 0.001$).

It will be observed that two values each in the thyrotoxicosis and myxedema groups fell well within the normal range. No obvious explanation was apparent; these values were evidently not due to technical errors since subsequent determinations yielded similar results.

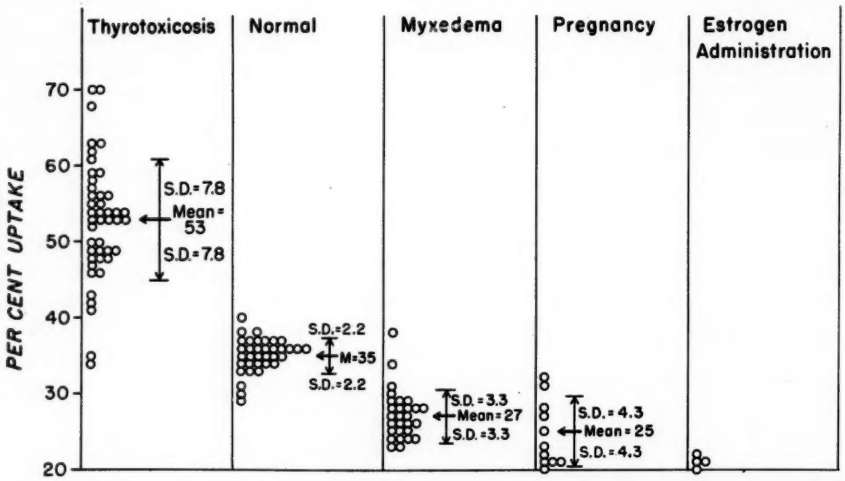

FIG. 1. Resin uptake of I^{131}-triiodothyronine in thyroid disease, pregnancy, and during estrogen therapy. All but 7 of the cases of thyrotoxicosis were untreated. The myxedema group included various degrees of hypothyroidism, including 5 cases following therapy for thyrotoxicosis and 4 cases of hypopituitarism.

The effects of treatment for thyrotoxicosis are illustrated in Figure 2. The elevated resin uptake values noted in active thyrotoxicosis fell after thyroidectomy, I^{131} therapy, or antithyroid drug therapy. The single patient with a persistently elevated uptake had residual thyrotoxicosis after I^{131} therapy.

The effect of markedly abnormal patterns of serum proteins is illustrated in Figure 3. Some of the uptakes, notably from the sera of 2 nephrotic patients, were well above the normal range.

Hypercapnea was studied because of the abnormally elevated uptake values reported with the erythrocyte method (2). Sera from 5 cases of CO_2 retention associated with pulmonary emphysema exhibited normal uptake values, with a mean of 37 per cent. In the present method, the serum be-

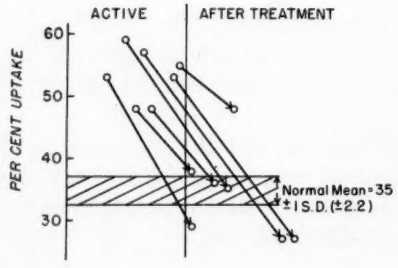

FIG. 2. Resin uptake of I^{131}-triiodothyronine in thyrotoxicosis, before and after treatment. Elevated uptake values in active thyrotoxicosis fell after treatment (thyroidectomy, I^{131}, and antithyroid drug therapy). The single instance of a persistently elevated value was observed in a patient with residual thyrotoxicosis after I^{131} therapy.

comes equilibrated with the room air, and this eliminates variation in CO_2 content as a source of error.

In 4 subjects receiving Lugol's solution, the resin uptake values were normal. Organic iodine dyes were likewise without effect. In 4 patients blood was drawn before and after intravenous pyelography, and before and after an intravenous cholangiogram; there was no detectable change in the resin uptake.

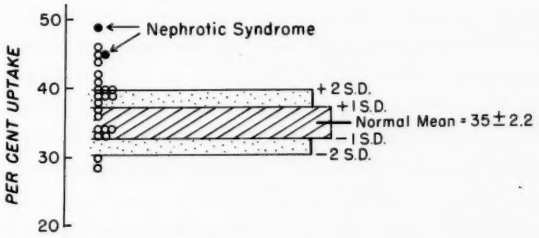

FIG. 3. Resin uptake of I^{131}-triiodothyronine in euthyroid subjects with marked abnormalities of serum proteins. These sera exhibited the most pronounced abnormalities in protein electrophoretic patterns observed in a routine laboratory.

DISCUSSION

The resin uptake of I^{131}-triiodothyronine was considered a satisfactory routine laboratory test of thyroid function, particularly useful in the presence of contamination with organic or inorganic iodine.

The previously described *in vitro* methods were less satisfactory in our hands. The erythrocyte uptake of I^{131}-triiodothyronine as determined by the method of Hamolsky, Freedberg and co-workers (1, 2) was found to be less convenient and less reproducible, requiring the measurement of a very small fraction of added radioactivity, which was subsequently adjusted to a higher numerical value by computation to a uniform hematocrit of 100 volumes per cent. The results could be affected by individual hematocrit variations.

The method originally described by Mitchell (3) for the resin uptake of I^{131}-thyroxine offered less diagnostic discrimination between categories than with the use of I^{131}-triiodothyronine. Subsequently Mitchell modified the test to employ I^{131}-triiodothyronine; the procedure is like the present one, except that the resin is obtained already embedded in plastic sponges by the supplier. The results of Mitchell *et al.* (4, 5) showed less diagnostic discrimination for thyroid diseases than the results with the present technique. The difficulty of preparation of different batches of resin sponges with identical properties presents an additional technical problem. Even if the sponge is manufactured in uniform batches, the resin-sponge method

seems to offer no advantage over the use of a measured amount of free resin.

Thyroxine in serum has been found to migrate with albumin, alpha-globulin, and pre-albumin on electrophoresis (6–11). The existence of a small moiety of "free" or unbound thyroxine has been postulated previously (6) and has been demonstrated by dialysis through cellophane (9, 10). Tracer amounts of I^{131}-labeled thyroxine added to serum yielded radioactivity in the dialysate which was identified as thyroxine chromatographically (9).

The uptake of tracer thyroxine or triiodothyronine from serum by the anion exchange resin, IRA-400, is believed to represent the resultant of the binding attraction of the resin and that of the serum proteins for triiodothyronine. A complete discussion of the interactions of the several binding proteins with thyroxine and triiodothyronine is beyond the scope of the present paper.

It appears probable that the thyroxine-binding alpha-globulin (TBG) is the protein of major physiologic and clinical significance in the binding of the thyroxine. Triiodothyronine is also bound by TBG, but thyroxine is bound more firmly, and readily displaces bound triiodothyronine from the complex (12). Thus, the unbound fraction of I^{131}-triiodothyronine is a sensitive indicator of the concentration of free thyroxine in serum. Free thyroxine is considered to be the physiologically active form of thyroxine (6) and its concentration presumably determines the effect upon the tissues.

In the present test the uptake of the tracer by the resin seems to bear an approximately inverse relationship to unoccupied protein binding sites. The precise relationship is complex. It should be mentioned that graded increments of thyroxine, added to normal or hypothyroid sera prior to resin uptake determinations, produced progressively elevated values (13).

The applicability of the present test to the great majority of sera likely to be received by clinical chemistry laboratories depends upon the assumption that the values will not be influenced appreciably by the more commonly encountered abnormalities in the pattern of serum proteins. Evidence to date (6, 13) has shown no marked abnormality of the serum proteins in thyroid disease. Consequently, in most sera likely to be examined for the presence of thyroid dysfunction, the resin uptake value may be expected to have its usual approximately inverse relationship to unoccupied protein binding sites. Under these circumstances, the resin uptake value will also reflect the total concentration of thyroxine in the serum. In contrast to PBI and BEI determinations, however, it is not affected by contamination with organic or inorganic iodine.

In pregnancy, and during administration of estrogen, the subjects are euthyroid, ordinarily with a normal basal metabolic rate, and with the concentration of free thyroxine presumed to be normal. However, the TBG or its binding capacity is increased. The percentage of added radioactivity taken up will be reduced, since the tracer becomes equilibrated with an increased number of binding sites on TBG. The reduced resin uptake with elevated PBI level seen in pregnancy corresponds to the delivery of an essentially normal quantity of hormone to the tissues. The phenomenon has been described and discussed by Beierwaltes and Robbins in their report on the familial increase in the thyroxine-binding sites in serum alpha-globulin (7).

In our study, the changes noted during estrogen therapy were considered similar to those in pregnancy, since TBG capacity is increased in each (16–18). The application of the test to prognosis in threatened abortion in early pregnancy remains to be explored in detail (19).

In cases of low TBG concentration or binding capacity, *e.g.*, in the nephrotic syndrome with a low serum PBI level (20), the tracer becomes equilibrated with a lesser number of binding sites. This results in an elevated resin uptake, even though the concentration of free thyroxine is normal and the patient is euthyroid.

Acknowledgments

The authors wish to express their gratitude for the cooperation of Dr. Sidney C. Werner; for the valuable technical assistance rendered by Robert Danziger and Philip Rosen; and for the assistance of Genevieve Covolo, Barbara Jones, Doris Snyder, Antoinette Schwob, Kathleen Ronayne and Edward Siegel, as well as Dr. Solomon Silver and Dr. David V. Becker, who helped procure sera.

Addendum

It has been suggested by Dr. Bill Nelson, Oak Ridge Institute of Nuclear Studies, that the data be expressed with reference to the pooled normal serum standard, which would be assigned a value of 1.00. In this way corrections would be made for variations in individual lots of the tracer. After initially finding reasonable correspondence with our results on aliquots of sera from the present series, Dr. Nelson has observed that some recent shipments of I^{131}-triiodothyronine have yielded considerably lower values for the normal standard with the same relative abnormalities in pathologic sera.

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EFFECT OF THYROTROPIN ON THE SERUM PROTEIN-BOUND IODINE LEVEL IN VARIOUS THYROID STATES (TSH-PBI TEST)*

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ABSTRACT

Single intramuscular injections of thyroid-stimulating hormone (TSH) were given to 22 normal subjects and to 67 patients with abnormal thyroid or anterior pituitary glands. Resulting changes in serum protein-bound iodine (PBI) concentration were measured. Among the normal subjects PBI concentrations had risen to maximal heights by the fifteenth hour after injection when the dose was 10 I.U. and by about the twentieth hour when the dose was 20 I.U. In both cases maximal elevations of PBI concentration were maintained beyond the twenty-fourth hour. Twenty-four hours after injection of 5, 10 or 20 I.U., the mean rises in PBI concentration for the normal subjects were 1.3 μg. $\pm$0.6 (S.D.), 2.2 μg. $\pm$0.6, and 2.8 μg. $\pm$1.0 per 100 ml., respectively. Among the patients, determination of rises in PBI concentration twenty-four hours after injection of 10 I.U. revealed that, with exceptions, responses were abnormally low in patients with diffuse thyroid disease whereas they were normal in patients with focal thyroid disease or pituitary failure. In some patients with nontoxic nodular goiter, the responses were abnormally high. Tests of this kind may be useful in distinguishing between a) Hashimoto's disease and other nontoxic nodular goiters, b) Graves' disease and toxic nodular goiter, and c) primary and secondary myxedema.

THE response of normal and diseased human thyroid glands to stimulation with exogenous thyrotropin (TSH), a diagnostic parameter of thyroid function, has been measured both by radioiodine technics and by determination of changes in the concentration of serum protein-bound iodine (PBI) (1–14). The data so obtained have contributed to formulation of theories of thyroid function and have been the basis for suggested diagnostic tests for diseases of the thyroid gland. In this paper some additional information is submitted on the effect of single doses of 5, 10 or 20 units of TSH on the serum PBI levels in normal people, and of single 10-unit doses on the PBI levels in patients with various thyroid and

Received September 24, 1960.

* Supported by a grant from the Louisiana Cancer Association and in part by an institutional research grant from the American Cancer Society, Inc. to the Division of Endocrine Research, Alton Ochsner Medical Foundation.

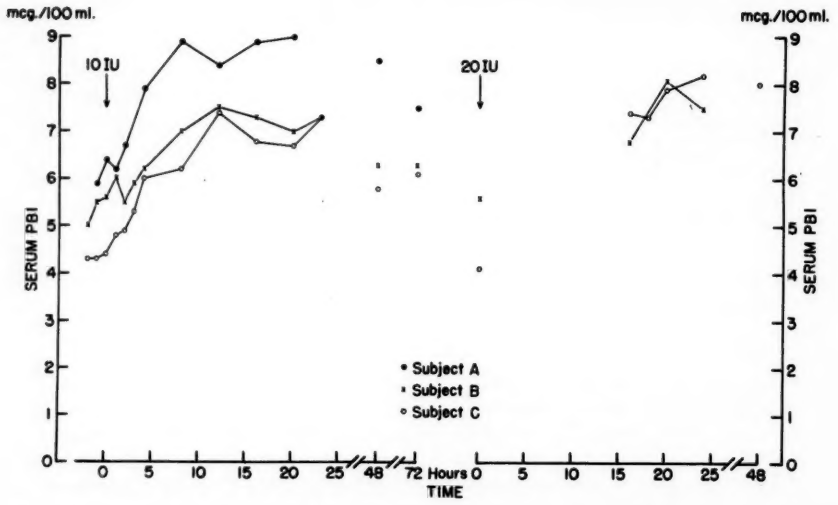

FIG. 1. Rise in serum protein-bound iodine concentration in normal subjects after single intramuscular injections of 10 and 20 I.U. of TSH.

pituitary diseases. We have been chiefly, but not exclusively, interested in the diagnostic value of this TSH-PBI test.

METHODS AND RESULTS

Serum PBI concentrations were determined according to the method of Barker *et al.* (15).[1] TSH[2] dissolved in 2 ml. of physiologic sodium chloride solution was administered intramuscularly.

Figure 1 shows the normal PBI response to injections of 10 and 20 I.U. of TSH over periods up to seventy-two hours. Three subjects received 10 I.U. and 2 received 20 I.U. The curves representing the PBI concentration reached plateaus at about fifteen hours after injection of 10 I.U. of TSH and at about twenty hours after injection of 20 I.U. of TSH, and remained there until at least twenty-four hours after injection. This information led us to select twenty-four hours as a convenient and biologically suitable interval

TABLE 1. RISE IN SERUM PBI LEVEL TWENTY-FOUR HOURS AFTER SINGLE INTRAMUSCULAR INJECTION OF TSH IN NORMAL SUBJECTS

Dose of TSH (I.U.)	No. of subjects	Serum PBI level (μg./100 ml.)		
		Mean	Range	Mean $\pm$ 2 S.D.
5	10	1.3	0.6–2.7	0 –2.6
10	10	2.2	1.3–3.0	1.1–3.4
20	4	2.8	1.9–4.1	0.8–4.8

[1] Determinations were made by the Bio-Science Laboratories, Los Angeles, California.
[2] Kindly supplied by Armour Pharmaceutical Company.

between injection of TSH and collection of blood for the final determination of PBI concentration.

The normal response of PBI concentration twenty-four hours after injection of 5, 10 or 20 I.U. of TSH is shown in Table 1. On the basis of these data we chose 10 I.U. of TSH as the standard dose for the TSH-PBI test. For this dose, the calculated normal range of serum PBI response is a rise of 1.1 to 3.4 μg. per 100 ml. This time-interval between injection of TSH and measurement of PBI response, and this specific dose of TSH, have been used by others in simi ar studies (8).

In Table 2 are listed the responses of patients with various thyroid and pituitary diseases. Diagnoses were established by the usual means. In some cases histologic diagnoses were also obtained.

In comparison with the PBI response in normal people, abnormally low responses were obtained in all patients with primary myxedema (12 cases), Hashimoto's disease (6 cases) and metastatic carcinoma of the thyroid (3 cases). The patients in the last group had undergone total thyroidectomy, were not receiving desiccated thyroid, and were not myxedematous. In 7 of 8 patients with toxic diffuse goiter, 2 of 5 with subacute thyroiditis, 1 of 3 with acromegaly, 2 of 7 with pituitary failure, 1 of 7 with toxic nodular goiter and 1 of 15 with nontoxic nodular goiter, the PBI responses were abnormally low. In 4 of 15 patients with nontoxic nodular goiter the responses were abnormally high. All other patients, including 1 with untreated carcinoma of the thyroid, responded normally.

COMMENT

The temporal relationship (Fig. 1) between the intramuscular injection of a single dose of TSH and the rise in serum PBI level was similar to that observed by Einhorn (11). The degree of normal response (Table 1) was almost the same as that reported by Lashmet *et al.* (8), but considerably less than that observed by Jefferies *et al.* (14).

We cannot explain the difference between our results and those of Jefferies *et al.*, but this difference directs attention to some defects of the test. Since the index used is a difference between two PBI determinations, errors may be additive. This source of error may be minimized by analyzing both samples of the pair at the same time. Then there is the apparently minor, but real, difficulty in transferring the hormone without loss from the vial in which it is dispensed into the syringe. These technical imperfections should be kept in mind when the results of this test are being interpreted.

Our patients with primary myxedema were a heterogeneous group. One had been made myxedematous with radioactive iodine. Three were nongoitrous cretins. Most of the others had undergone thyroidectomy for various reasons. Thus the data presented here may not completely portray the response to the test of adults with spontaneous primary myxedema.

It has been suggested many times that a TSH-PBI test or a test based on the ability of TSH to raise the thyroidal uptake of radioactive iodine (TSH-I^{131} test) can distinguish between primary and secondary myxedema. The results in a case of undoubted severe postpartum pituitary necrosis

TABLE 2. EFFECT OF SINGLE INTRAMUSCULAR INJECTIONS OF 10 I.U. OF TSH ON THE SERUM PBI LEVEL IN PATIENTS WITH VARIOUS THYROID AND PITUITARY DISORDERS

Patient		Serum PBI level (μg./100 ml.)		Comments
No.	Sex & age	Before TSH	Rise after TSH	
		Primary Myxedema		
1	M 18	0.6	−0.3	After thyroidectomy for thyrotoxicosis.
2	M 25	0.9	0.5	Cretin, nongoitrous.
3	M 27	1.0	−0.1	Cretin, nongoitrous.
4	F 26	1.0	0.6	After thyroidectomy for Hashimoto's dis.
5	F 38	1.8	0.5	After I^{131} therapy for thyrotoxicosis.
6	F 32	3.6	−0.4	After thyroidectomy for ca. of thyroid; Rx with desicc. thyroid
7	F 37	0.9	0.2	After thyroidectomy for Hashimoto's dis.
8	F 52	1.2	0.2	Postoperative.
9	F 55	1.1	0.2	After thyroidectomy for thyrotoxicosis.
10	F	1.1	0.3	
11	F	2.1	0.0	After thyroidectomy for thyrotoxicosis.
12		2.1	0.0	Cretin, nongoitrous.
		Pituitary Failure		
13	M 42	2.0	2.0	Chromophobe adenoma, 10 yrs.
14	M 72	2.4	2.3	Idiopathic, 15 yrs.
15	F 19	3.6	1.5	Hypothalamic tumor, 10 yrs.
16	F 28	2.8	1.6	Sheehan's syndrome.
17	F 33	4.2	1.0	Sheehan's syndrome, treated with desicc. thyroid.
18	F 52	2.8	0.8	Sheehan's syndrome, 15 yrs.
19	F 55	2.5	2.7	Sheehan's syndrome, 20 yrs.
		Acromegaly		
20	F 37	7.5	3.4	Acromegaly, inactive.
21		4.7	1.4	Acromegaly, active.
22	F 52	5.8	0.8	Acromegaly, inactive.
		Solitary Nodule of Thyroid		
23	M 22	7.8	1.6	Adenoma of thyroid.*
24	M 34	5.9	2.1	Fetal adenoma.*
25	M 52	5.2	4.3	Colloid cyst of thyroid.*
26	F 28	5.7	1.2	
		Nontoxic Multinodular Goiter		
27	M	5.4	4.0	
28	F 22	6.8	2.6	
29	F 27	6.5	0.8	
30	F 32	7.4	3.8	Colloid goiter.*
31	F 33	5.8	2.3	
32	F 57	7.0	2.2	Adenomatous goiter.*
33	F 58	5.1	1.2	
34	F 58	7.5	3.3	Colloid nodular goiter.*
35	F 29	6.4	4.5	Colloid nodular goiter.*
36	F	6.1	1.6	
37	F	6.2	2.5	

* Histologic diagnosis.

TABLE 2. (*continued*)

Patient		Serum PBI level (μg./100 ml.)		Comments
No.	Sex & age	Before TSH	Rise after TSH	
		Hashimoto's Disease		
38	F 12	5.5	−0.7	*
39	F 12	6.2	0.2	*
40	F 17	2.3	0.9	*
41	F 35	1.5	0.5	*
42	F 37	3.9	0.2	*
43	F 54	4.4	0.5	*
		Subacute Thyroiditis		
44	M 38	8.5	1.8	*
45	F 21	5.8	−0.1	*
46	F 41	11.5	0.4	
47	F 47	6.5	3.1	Clinically, only part of gland involved.
48	F 48	11.0	1.4	
		Toxic Nodular Goiter		
49	F 48	10.0	1.1	Initial PBI value 3 days before TSH.
50	F 58	11.8	1.6	
51	F 58	8.8	0.5	
52	F 60	12.0	2.2	
53	F 62	8.9	1.4	
54	F 65	9.6	2.3	
55	F 67	11.0	1.6	
		Toxic Diffuse Goiter		
56	M 17	11.7	0.2	
57	F 23	14.5	−1.5	
58	F 26	17.0	1.0	
59	F 29	19.2	0.8	
60	F 33	9.4	−0.2	
61	F 34	15.5	0.2	
62	F 58	12.6	0.0	
63	F 65	11.0	2.0	
		Carcinoma of Thyroid		
64	M 42	6.0	2.0	Well differentiated carcinoma.*
		Postoperative Metastatic Carcinoma of Thyroid		
65	M 59	4.2	−0.1	No treatment with desicc. thyroid.
66	M 66	4.5	−0.2	No treatment with desicc. thyroid.
67	F 58	6.8	0.6	No treatment with desicc. thyroid.

(No. 17, Table 2) show that a low response to the TSH-PBI test, as here described, does not eliminate the diagnosis of anterior pituitary insufficiency. Perhaps multiple injections of TSH for a period of several days would have raised the PBI concentration to a greater degree. Jefferies

et al. (14) suggested that the TSH-I^{131} test is more reliable than the TSH-PBI test in distinguishing between the two forms of myxedema. According to these authors, the response in the TSH-PBI test requires the presence in the thyroid of already formed thyroid hormone, whereas the TSH-I^{131} test does not. However, Skillern and Evans (7) noted unresponsiveness even to the TSH-I^{131} test among some patients with anterior pituitary insufficiency. Sheehan and Summers' observation (16) of almost total absence of thyroid tissue in some patients with postpartum pituitary necrosis may afford an explanation of this phenomenon.

Several investigators (3, 9) have showed that the overactive thyroid is abnormally resistant to the action of injected TSH. As far as we can determine, these observations by others did not encompass both Graves' disease and nodular goiter with hyperthyroidism. Our experience seems to indicate that only patients with Graves' disease show an abnormally low PBI response to injected TSH. As a group, our patients with nodular goiter and hyperthyroidism responded normally. One might conjecture that they possessed reactive internodular tissue.

We are not aware of any previous demonstration that the serum PBI response to injected TSH is abnormally low in Hashimoto's disease. This observation is, however, consistent with the observation of Skillern *et al.* (6) that, in this disease, TSH raises the thyroidal uptake of I^{131} little if at all. These authors suggested that a TSH-I^{131} test might be used to differentiate Hashimoto's disease from other nontoxic nodular goiters. The same may be true of the TSH-PBI test.

Of great interest but of unknown significance were the abnormally high PBI responses in 4 patients with nontoxic nodular goiter. No other patients showed this hyper-reactivity.

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SOME PATHOLOGIC CORRELATIONS OF OVARIAN STROMAL HYPERPLASIA*

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ABSTRACT

Six hundred autopsy cases were collected of adult women whose ovaries had been examined microscopically. Half these women had ovarian stromal hyperplasia, and half did not. Obesity, arteriolar nephrosclerosis, endometrial overgrowths and uterine leiomyomas were significantly more frequent in the women with ovarian stromal hyperplasia. The incidence of diabetes was related to the incidence of obesity, hypertension and nephrosclerosis, but these relationships were independent of the ovarian status. The occurrence of generalized arteriosclerosis, myocardial infarction and most other diseases was not significantly different in the two ovarian groups. Diverticulosis coli was more common in the group without ovarian hyperplasia.

OVARIAN cortical stromal hyperplasia accompanies most cases of endometrial hyperplasia or cancer, mammary adenocystic disease or carcinoma, ovarian hyperthecosis, and pulmonary adenocarcinoma in women (1, 2). Considerable indirect or circumstantial pathologic evidence points to ovarian "estrogenic stroma" as the major source of estrogenic hormones in adult women (3). Target organs of estrogenic ovarian stimuli, such as the vaginal epithelium, uterine cervix, endometrial glands and mammary ducts usually show histologic evidence of active stimulation when ovarian stromal hyperplasia is present. Furthermore, the cells of the ovarian cortical stroma are known to convert radioactive acetate biochemically *in vitro* into a variety of radioactive estrogens (4). The collected data, interpreted from histologic, cytologic, pathologic and biochemical viewpoints, indicate that a continuous secretion of estrogen occurs from the hyperplastic ovarian stroma.

There is evidence from various types of investigation that, compared to men, women are spared to a certain degree from the development of advanced arteriosclerosis and its complications (5–7). A leading theory ascribes some protective action to estrogen. Indeed, estrogen has been used in therapeutic trials on arteriosclerotic men (8). It was thought of

Received July 18, 1960.

* Aided in part by Grant C-4017 (C2) from the National Institutes of Health, United States Public Health Service.

interest in this context to investigate the range of pathologic conditions that occur in the presence or absence of ovarian stromal hyperplasia. In the present study the diseases found at autopsy were identified in two groups of women, predominantly postmenopausal, who either possessed or lacked ovarian tissue showing histologic evidence of active synthesis of estrogen.

MATERIALS AND METHODS

Six hundred autopsy cases of adult women whose ovaries had been examined microscopically were collected from two Boston institutions, the New England Deaconess Hospital and Massachusetts Memorial Hospitals. The material was otherwise unselected, except that an attempt was made to match the women with and without ovarian stromal hyperplasia by age groups. The Deaconess cases totaled 494—238 with, and 256 without ovarian stromal hyperplasia. The Massachusetts Memorial cases totaled 106—62 with, and 44 without ovarian stromal hyperplasia.

TABLE 1. AGE GROUPS OF WOMEN WITH OR WITHOUT OVARIAN STROMAL HYPERPLASIA

Age group (yrs.)	Ov. stromal hyperplasia	Controls (no hyperplasia)
21–35	17	17
36–40	10	10
41–50	36	44
51–60	81	79
61–70	92	80
71–80	52	53
81–91	12	17
	300	300

The two series are listed by age groups in Table 1. No significant differences were found, in the age distributions, using the chi-square statistical method (9). The population under study was derived from women dying in two general hospitals that serve mostly middle-income groups of Americans, predominantly white Protestant native-born adults. Most of the autopsies were performed between 1940 and 1955.

The criteria for a diagnosis of ovarian cortical stromal hyperplasia were: 1) nodular thickenings of the superficial, closely packed, spindle-shaped ovarian stromal cells; 2) superficially located nodules of this hyperplastic tissue forming a layer at least 1 mm. thick, and 3) clear spaces between the cells in the hyperplastic stroma, indicative of varying amounts of lipoid accumulation—so-called thecomatosis. By contrast, postmenopausal ovaries considered to show no such hyperplasia microscopically had either a thinner superficial stromal layer, or no stromal nodules, or both. Atrophic ovaries were largely replaced by a relatively acellular hyalinized collagenous tissue (10).

To identify the other diseases found at autopsy, all the major gross and microscopic diagnoses were collected after histologic evaluation of the ovaries was completed. The autopsy diagnoses were then listed and tabulated, in order to compare the two groups. Particular attention was paid to other endocrine disorders such as diabetes mellitus, and to obesity, hypertension, vascular disease and cancer. All diagnostic categories for the two groups were compared statistically with the use of the chi-square method, and val-

TABLE 2. MAJOR DISEASES FOUND IN WOMEN WITH OR WITHOUT OVARIAN STROMAL HYPERPLASIA

	300 Cases of ov. stromal hyperplasia	300 Controls
Generalized arteriosclerosis	191 (64%)	178 (59%)
Arteriolar nephrosclerosis	165 (55%)	100 (33%)
Obesity	121 (40%)	74 (25%)
Hypertensive disease	107 (36%)	82 (27%)
Diabetes mellitus	93 (31%)	73 (24%)
Leiomyoma uteri	98 (33%)	72 (24%)
Cholecystitis or lithiasis	81 (27%)	77 (26%)
Endometrial polyp or hyperplasia	72 (24%)	48 (16%)
Myocardial infarct	51 (17%)	48 (16%)
Adenomatous goiter	38 (13%)	23 (8%)
Pyelonephritis	30 (10%)	46 (15%)
Fatty liver	27 (9%)	31 (10%)
Cystitis	22 (7%)	28 (9%)
Colonic polyps	21 (7%)	20 (7%)
Diverticulosis coli	21 (7%)	39 (13%)

ues of $P<0.05$ (which correspond to relations that would occur by chance less than once in twenty times) were considered significant. Slide-rule calculations to three figures were used.

RESULTS

Certain statistically significant differences were found on comparison of the two groups with regard to the most frequently diagnosed pathologic conditions (Table 2). Arteriolar nephrosclerosis ($P<0.001$), obesity ($P<0.001$), endometrial polyp or hyperplasia ($P<0.025$), and leiomyoma uteri ($P<0.025$) were significantly more common in association with ovarian stromal hyperplasia than in the controls without hyperplasia. Diverticulosis coli was significantly more frequent ($P<0.05$) in the absence of ovarian stromal hyperplasia.

There were no significant differences between the two groups with regard to the other commonly observed pathologic conditions and the rarer abnormal conditions (Table 3) when tested by chi-square calculations. In addition to the diagnoses tabulated, cardiovascular rheumatic, arteriosclerotic and hypertensive diseases were found with equal frequency in the

TABLE 3. RARER CONDITIONS IN WOMEN WITH OR WITHOUT OVARIAN STROMAL HYPERPLASIA

	300 Cases of ov. stromal hyperplasia	300 Controls
Adrenocortical hyperplasia	15 (5%)	9 (3%)
Pituitary adenoma	9 (3%)	3 (1%)
Chronic thyroiditis	5 (2%)	9 (3%)
Adrenocortical adenoma	4 (1%)	10 (3%)
Hydronephrosis	5 (2%)	14 (5%)
Adrenocortical atrophy	4 (1%)	8 (3%)
Ulcerative colitis	1 (1%)	7 (2%)

two groups. Urinary-tract diseases of twenty-three diagnostic types were also compared, without demonstrating any differences. Gastric, hepatic, biliary, thyroid, adrenal, pituitary and parathyroid abnormalities were also about equally common in both groups.

Cancer occurred in 98 control cases and in 77 women with ovarian stromal hyperplasia. This is not a significant difference. Thirty cancer sites were compared. In carcinoma of the uterine cervix, 10 women had ovaries with no stromal hyperplasia, and only 1 had ovaries with hyperplasia. The significant predominance ($P < 0.025$) of women without hyperplastic ovaries has already been reported in a study of host factors in a much larger series of cases of cervical cancer (11). For the other sites investigated there were no discrepancies, but only 2 endometrial carcinomas were found—one in each group.

Correlations of various abnormalities showed significant relationships between obesity and arteriolar sclerosis, obesity and hypertensive disease, and obesity and diabetes, independent of the ovarian status ($P < 0.001$). The single exception was that cardiac hypertrophy and obesity were not correlated significantly in the group without ovarian stromal hyperplasia.

Diabetes mellitus was significantly correlated with hypertensive disease and arteriolar nephrosclerosis ($P < 0.005$), but the diabetic subgroups with or without ovarian stromal hyperplasia did not show statistical evidence of an increased incidence of hypertensive lesions or nephrosclerosis. In the group with ovarian stromal hyperplasia there were no significantly altered incidences either of diabetes mellitus, hypertensive disease or both, as compared to the entire population studied or to the group without ovarian hyperplasia. Endometrial hyperplastic changes and leiomyomas did not occur together with significant frequency, as compared to their occurrence individually.

DISCUSSION

Within the limits of the postmortem material investigated and the statistical tests employed, obesity, arteriolar sclerosis, endometrial overgrowth and uterine leiomyomas were found in women with ovarian stromal hyperplasia significantly more often than in women without such hyperplasia. Hypertensive cardiac disease, diabetes mellitus, generalized arteriosclerosis and a variety of other pathologic conditions were not correlated in incidence with the ovarian status. Diverticulosis coli was significantly more common in the absence of ovarian stromal hyperplasia.

Obesity, hypertensive disease, diabetes and arteriolar sclerosis were demonstrated to have statistical interrelationships that were independent of ovarian stromal hyperplasia. Such already widely accepted disease relationships are clearly distinguishable by statistical analysis from the

correlations found between ovarian stromal hyperplasia, arteriolar nephrosclerosis, obesity and uterine overgrowths. Persons with the latter syndrome include the "burgeoning women" of gynecological interest (12), and the female "endocrine hypertensives" of Schroeder and Davies (13). The tendency toward development of endometrial cancer in this group was noted previously by Sommers and Meissner (14).

At present it is not clear whether androgenic and progestinic hormones are produced in effective quantities by hyperplastic ovarian stromal tissue. Certainly the major target organs of the ovary do not show changes attributable to such hormones in most cases of ovarian stromal hyperplasia. In the present study the important intertwined endocrine functions of the adrenal cortex could not be accurately estimated. However, estrogens from the hyperplastic ovarian stroma evidently did not protect these postmenopausal women from the development of arteriosclerosis or myocardial infarction. Quantitative studies of the secretion of estrogen by hyperplastic ovarian stroma perhaps would help to unravel this apparent paradox.

Acknowledgment

Appreciation is expressed to Dr. Edward L. Pelikan for advice and assistance with the statistical evaluations.

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Letters to the Editor

IMMUNOLOGIC PROPERTIES OF URINARY PREPARATIONS OF HUMAN MENOPAUSAL GONADOTROPINS, WITH SPECIAL REFERENCE TO PERGONAL*

To the Editor:

The purpose of this communication is to report the immunogenic properties of purified preparations from human menopausal urine (HMG). The preparations used were: Pergonal 22, Pergonal 23 (1)[1], HMG 24[2] and HMG J5[3].

Pergonal 22 is a purified menopausal urinary gonadotropic extract, containing 0.8 mg. of sodium thymonucleate per 1.0 mg. of extract. It was prepared by kaolin adsorption followed by Permutit column chromatography. The biologic activity was 15 mg.-equivalents of the international reference preparation for human menopausal gonadotropin (IRP-HMG). The mouse uterus test was used in all cases for standardization of biologic activity of gonadotropic extracts. IRP-HMG was always used as a reference standard.

Pergonal 23 was prepared from another pool of menopausal urine purified by the same methods as for Pergonal 22, but containing no sodium thymonucleate. The biologic activity of this preparation was 22 mg.-equivalents of IRP-HMG.

HMG 24 was prepared by kaolin adsorption with no subsequent Permutit column chromatography, and served as the IRP-HMG.

HMG J5 was prepared by Permutit chromatography, but no kaolin adsorption. The biologic activity was 24 mg.-equivalents of IRP-HMG.

Antiserum was prepared as follows: A 25-mg. sample of Pergonal 22 (14 mg. of gonadotropic extract+11 mg. of sodium thymonucleate) was emulsified in 1.5 ml. of complete Freund Adjuvant (Difco Laboratories). Of this mixture, 0.5 ml. was injected into the thigh of each of 2 female

* This study was supported in part by a grant from the Israel Foundation Trustees.

[1] Kindly donated by Dr. P. Donini, Instituto Farmacologica "Serono."

[2] Kindly supplied by Dr. Bangham, Department of Biological Standards, Medical Research Council, Mill Hill, London.

[3] A gift of Dr. S. G. Johnson, Statens Serum Institute, Copenhagen.

2.5-Kg. rabbits. Three injections were given at fortnightly intervals. Pre-immunization sera contained no precipitating antibodies against either Pergonal 22 or 23, nor did they inhibit the biologic activity of gonadotropic extracts. Regular weekly bleedings of animals were started after the third injection. Bleedings were continued for five weeks and all the samples were pooled.

The pooled antiserum was tested for the presence of antipergonal antibodies by the precipitin test (2). It was found that as little as 5 γ of Pergonal 22 (equivalent to the biologic activity of 0.075 mg. of IRP-HMG) could be detected. The supernatants and the precipitates obtained by adding different concentrations of gonadotropic extracts to constant volumes of antiserum were then analyzed. The supernatants were assayed biologically for residual gonadotropic activity. Complete neutralization of biologic activity in the antibody excess zone was noted in all extracts (Pergonal 22 and 23, HMG 24, HMG J5). In the equivalence zone the biologic activity was neutralized completely for HMG 24 and reduced by 90 per cent for Pergonal 22 and 23. HMG J5 could not be tested in the equivalence zone due to lack of sufficient material. Increasing biologic activity was correlated with increasing quantities of antigen in the antigen excess zone.

The quantity of precipitate was estimated by two methods: 1) micro Kjeldahl determination of nitrogen, and 2) extinction at 280 mμ of the precipitate dissolved in NaOH and neutralized by HCl. Agreement was obtained by the two methods.

When precipitin curves (Fig. 1, optical density of dissolved precipitates) of Pergonal 22 and 23 were compared to that of HMG 24 it was found that the equivalence zone for HMG 24 was at 15 mg. of the preparation, whereas for Pergonal 22 and 23 it was at 0.8 mg. and 1 mg. respectively. Figure 2 presents the same data as in Figure 1, except that the abscissa is expressed in terms of biologic activity. It can be seen that the immunologic activity is parallel to the biologic activity rather than to the weight of the different preparations.

Several experiments were conducted to characterize Pergonal, *viz*, ultracentrifugation, electrophoresis, and gel-diffusion.

A sample of Pergonal 23 (5 Gm. per liter of 0.05 *M* phosphate buffer, pH 6.5) was sedimented in the ultracentrifuge. One slightly asymmetric peak was observed with a sedimentation constant of $S_{20,w} = 2.0$ Svedberg units. This slight asymmetry indicated that Pergonal might not be homogenous.

Fractionation by electrophoresis on polyvinyl choride particles (Geon, Goodrich Company) in borate buffer or veronal buffer at pH 8.5 and 0.05 ionic strength, 2° C., 25 m.a. and 440 V., was carried out for fifteen hours.

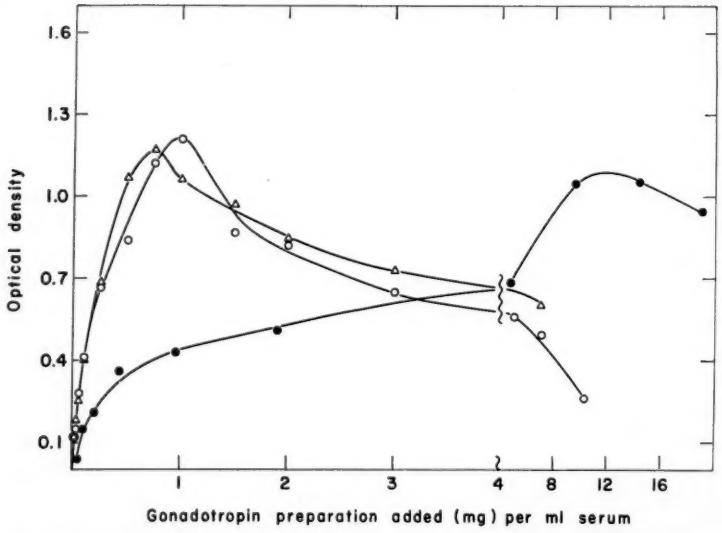

FIG. 1. The optical density, at 280 mμ, of neutral solutions of precipitates (dissolved in 0.1 *N* sodium hydroxide and neutralized with 1 *N* hydrochloric acid) obtained by the addition of Pergonal 22 (△), Pergonal 23 (○) and HMG 24 (●) to the antiserum to Pergonal 22, as a function of the amount of the precipitant.

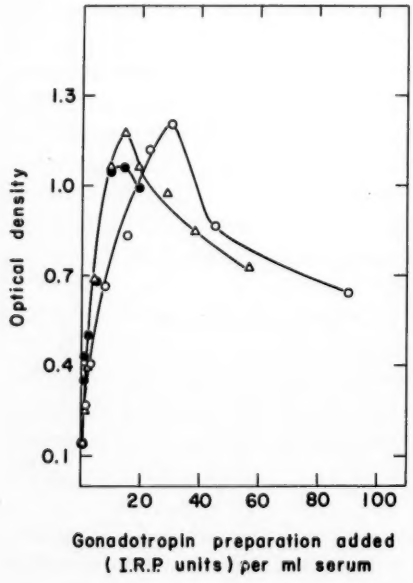

FIG. 2. The optical density, at 280 mμ, of neutral solutions of precipitates obtained by the addition of Pergonal 22 (△), Pergonal 23 (○) and HMG 24 (●) to the antiserum to Pergonal 22, plotted as a function of the biologic activity expressed in the international reference preparation of human menopausal gonadotropin.

The Geon block was cut crosswise into 24 segments (1 cm. each). The segments were eluted with distilled water and the eluates were tested for: (a) protein content, by the Folin biuret procedure (3); (b) immunologic reactivity by the precipitin technic, and (c) biologic activity by mouse uterine and ovarian weight technics. The material moved towards the anode. Two or three peaks of protein could be demonstrated when the different eluates were analyzed for their protein contents. Immunologic reactivity by precipitin tests was shown in the eluates of the segments composing the slowest moving peak, as well as the central peak. Biologic activity could be detected only in the eluates of the segments composing the central peak. Thus it seems that Pergonal is made up of more than one antigen, and that at least one of these antigens has biologic activity.

In gel-diffusion experiments Pergonal was allowed to react with its antiserum. Two precipitin lines were observed.

SUMMARY

The immunologic studies described here indicate: 1) that gonadotropins are immunogenic; 2) that the biologic and immunologic activities in the gonadotropic extracts tested were parallel, and 3) that Pergonal contains more than one immunogenic component. Further studies are necessary to characterize the unspecific proteins in Pergonal which are not related to the biologically active molecule.

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THE ROLE OF ANDROGENIC HORMONES IN HUMAN SEXUAL BEHAVIOR

To the Editor:

The following conclusion is suggested to the reader of the interesting paper by Schon and Sutherland (*J. Clin. Endocrinol. & Metab.* **20**: 833, 1960): Libido and sexual activity of mature women is chiefly dependent on the androgenic hormones of the adrenals. Formulated in this way, the statement has, like all generalizations, a core of truth and insofar possesses a definite validity. Such generalizations, however, are almost equally well calculated to obscure the facts of the matter, in that they tend to suppress opposing data. Other investigations have been undertaken in an attempt to evaluate the role played by hormonal function in human sexual behavior. Perhaps it will be helpful in clarifying the foregoing assertion to glance at some experience gained in these related studies.

Our own series of cases subjected to adrenalectomy or hypophysectomy yielded findings which point in the direction indicated by Schon and Sutherland or by Waxenberg, Drellich and Sutherland (1). There were, however, noteworthy exceptions: For instance, a 46-year-old woman with carcinoma of the breast who had undergone not only ovariectomy and adrenalectomy but finally hypophysectomy (with remission following each intervention) reported that she felt no alteration in the sexual sphere following any one of the three operations. Another 41-year-old woman who was interviewed some months after successful hypophysectomy reported likewise that she had not experienced any detectable decline in libido. Such observations show clearly that the sexual behavior of mature women cannot depend directly and exclusively on the production of hormones.

In patients suffering from Cushing's syndrome, as described recently by Furger (2), there was no correlation between changes in sexual behavior and increased production of androgens as measured by the urinary output of 17-ketosteroids. Women who were happily married and who enjoyed sexual relations before their illness, experienced in the course of Cushing's syndrome a total loss of libido, despite a massive increase in the production of hormones by the adrenal cortex. After successful bilateral adrenalectomy, both libido and the former capacity to achieve sexual gratification were fully restored. Especially interesting is the observation that, postoperatively, 2 of these patients enjoyed a fuller intimacy in their relations with their sexual partner than before the illness. Still other patients experienced, during Cushing's syndrome, a pronounced increase of libido with strong sexual impulses, but no increase in the urinary output of 17-

ketosteroids. In our series of women with Cushing's syndrome, neither increase nor diminution of libido could be convincingly correlated with the functioning of the adrenals as determined by ordinary methods of hormone analysis. On the other hand those women who, prior to the illness, were sexually more active than others, were likely to experience in the course of Cushing's syndrome an increase of libido, whereas those who formerly were relatively disinterested sexually, lost their libido completely.

The findings in patients with the adrenogenital syndrome lend equally small support to the general assertion that the androgens of the adrenal cortex directly account for the presence or strength of sexual libido. Züblin (3) examined a number of women suffering from the adrenogenital syndrome. In spite of the flooding of the organism with androgens, the psychosexual development of these women, instead of being enhanced, was more often restricted and infantile. Allen (4) also, in his study of women with the adrenogenital syndrome and virilism, frequently observed a decrease of the normal libido which returned to the previous level after adrenalectomy.

The effects of androgens in persons with hypogonadism are well known. In these patients androgens may provoke a wakening of sexuality parallel to somatic maturation. But whether this is manifested as a consciously experienced increase in libido or merely as heightened anxiety and loss of self-confidence depends upon the whole personality.

The administration of androgens in large doses frequently increases the libido of mature women, but this effect on sexual behavior is not uniformly observed, and also can vary in kind from case to case. One woman may experience an agreeable and consciously affirmed increase of libido, whereas another feels only itching and distressing sensations in the genital region, which are not interpreted as sexual. There is a broad scale of transition between these extremes. Thus the result of such hormonal therapy depends only partially upon the hormone itself and the level of dosage; the personality of the woman involved, her age, mental health, psychosexual maturity and general physical condition, and the existing erotic situation play an equally decisive role. There are as yet no detailed observations on a sufficiently large series in a study giving adequate consideration to all of these factors. Apart from this lack of well documented studies, psycho-endocrinologic investigations have always met with disappointment when attempts have been made to establish a simple correlation between a specific hormonal effect and a psychic process. The work of Bleuler (5) and his school has shown this impressively. What emerges from these studies is that we cannot lose sight of personal and individual factors in the functional interplay between psychic and hormonal processes,

if we wish to avoid doing violence to the facts in favor of what purports to be a universally valid rule.

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The Endocrine Society

The 1961 Annual Meeting

The Forty-Third Annual Meeting of the Endocrine Society will be held in the Hotel Biltmore, New York, N. Y., Thursday, Friday and Saturday, *June 22, 23 and 24, 1961.*

The Scientific Sessions will be held from 9:00 a.m. to 5:00 p.m. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 23, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make their hotel reservations immediately. The Biltmore will hold 300 bedrooms for members until May 1, 1961, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 26 for the American Medical Association and other meetings, it is imperative that your reservations be made from June 26 on, directly through the A. M. A. Housing Bureau. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1961.

Final program, membership card and advance registration forms will be sent on May 1, 1961 to members whose current dues have been paid.

The 1961 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service

Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Thirteenth Postgraduate Assembly in Endocrinology and Metabolism

Under the Co-Sponsorship of The Endocrine Society
and The National Institutes of Health
Bethesda, Maryland
October 2–6, 1961

A comprehensive review of clinical endocrine problems and current research activity in these areas will be presented. For further information, write to: Dr. Roy Hertz, National Institutes of Health, Building 10, Bethesda 14, Maryland. The fee will be $100.00 for physicians, with a reduction to $30.00 for Residents and Fellows. Enrollment limited to 100.

The American Goiter Association

THE 1961 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in the Warwick Hotel, Philadelphia, Pennsylvania, *May 3–6, 1961.* Arrangements are being made for a Clinical Day in the Philadelphia Hospitals on May 3. Scientific papers will be presented in the Warwick Hotel on May 4, 5 and 6. For further information address the Secretary, John C. McClintock, M.D., 702 Madison Avenue, Albany 8, N.Y.

CLINICAL DAY PROGRAM

May 3, 1961

Morning session at the Hospital of the University of Pennsylvania, 34th and Spruce Streets, (Alumni Hall).

Operative Clinics:

8:30–10:30 a.m. "Surgery of the Thyroid Gland": *Drs. Ravdin, Rhoads, Johnson and associates.*

Panel Discussion:

10:30–12:00 noon "The Problem of Nodular Goiter"

Moderator: *Dr. Edward Rose.*

Panel: *Dr. William Ehrich, Dr. Robert Robbins and Dr. Julian Johnson*

(Question and Answer period)

Luncheon 12:00–1:45 p.m.

Afternoon Session at Jefferson Medical College, 1025 Walnut Street, (McClellan Hall).

Panel Symposium:

1:45–3:10 p.m. "Non-Toxic Goiter: Fundamental and Clinical Aspects."

Moderator: *Dr. Karl E. Paschkis*

Panel and Topic:

Dr. J. J. Rupp, Abnormal Thyroid Secretion in Euthyroidism.

Dr. A. M. DiGeorge, The Goitrous Cretin; Defects and Treatment.

Dr. A. M. Bongiovanni, Thyroid Auto-Immunity and Goiter.

Dr. S. A. D'Angelo, Role of the Pituitary in Goitrogenesis.

(Question and Answer period)

Intermission: 3:10–3:30 p.m. Refreshments

Presentations:

3:30–4:20 p.m. "Validity and Limitation of Clinical Methods for Evaluation of Thyroid Function."

Dr. S. Kramer, Radioiodine in Diagnosis.

Dr. T. Danowski, Plasma PBI and Lipids in Thyroid Disease.

Discussants: *Dr. A. Reventos* and *Dr. N. Schneeberg*

4:20–4:40 p.m. "Thyroid Function in Normal and Abnormal Reproductive States."

Dr. A. Rakoff

Discussant: *Dr. Mary Dratman*

4:00–5:00 p.m. "Diagnosis and Treatment of Parathyroid Disease."

Dr. John Blizzard

Discussant: *Dr. L. C. Mills*

SCIENTIFIC PROGRAM

May 4, 5 and 6, 1961

Thursday, May 4—9:00 a.m.

1. Metabolism of Thyroid and Thyroxine Iodine in Rats and Human Beings, *L. Van Middlesworth and Anthony J. Whitehead.*
2. Structure—Activity Relations as Indicated by Responses to Different Routes of Administration of 3,5,3′-Triiodothyropropionic Acid, L-Thyroxine, and L-Triiodothyronine, *Neil R. Stasilli, Robert L. Kroc and Paul J. Nemith.*
3. Inhibitory Effects of Thiouracil and Propylthiouracil on the *in Vitro* Deiodination of Thyroid Hormones, *Lewis E. Braverman and Sidney H. Ingbar.*
4. Direct Demonstration of Free Thyroxine in Serum, *Kenneth Sterling and Milton Tabachnick.*
5. Thyroxine-Binding Protein Studies in Normal Male Adolescents, *David J. Dreyer and Evelyn B. Man.*
6. Studies on the Thyroxine Binding Capacity of Plasma Using the Erythrocyte Uptake of *l*-Triiodothyronine Labelled with Radioactive Iodine, *Paul G. Walfish, Amy Britton, Robert Volpé and Calvin Ezrin.*
7. Studies Concerning the Placental Transfer of Antithyroid Antibodies and Cretinism, *R. W. Chandler, M. Kyle, W. Hung and R. M. Blizzard.*

Thursday, May 4—2:00 p.m.

8. The Value of Needle Biopsy of the Thyroid Gland in the Management of Lymphocytic (Hashimoto's) Thyroiditis, *Oliver H. Beahrs and Lewis B. Woolner.*
9. Effect of Iodides on Hashimoto's Thyroiditis, *Jaime Paris, Welby N. Tauxe, Lewis B. Woolner and Robert C. Bahn.*
10. The Relation of Thyroid Autoimmunity to Round-Celled Infiltration of the Thyroid Gland, *R. O. K. Schade, S. G. Owen, G. A. Smart and R. Hall.*
11. Autoantibody Titers in Struma Lymphomatosa: Pathologic Correlations, *D. E. Senhauser and J. B. Hazard.*
12. Functional Changes in Experimental Thyroiditis, *S. G. Jenkins, Jr. and C. G. Thomas, Jr.*
13. Hyperthyroidism Associated with Struma Ovarii: A Rare Surgical Challenge, *Edward S. Judd.*
14. The Late Results of Radioactive Iodine Treatment of Hyperthyroidism in Children and Adolescents: A Preliminary Report of Sixty Cases, *Paul Starr, Boris Catz, Donald Petit, Leon Oettinger and Henry Jaffe.*
15. Thyroid Nodules Occurring Late After Treatment of Thyrotoxicosis with Radioiodine, *Glenn E. Sheline, Stuart Lindsay, Kenneth R. McCormack and Maurice Galante.*

Friday, May 5—8:30 a.m.

16. Cytochrome Oxidase, Catalase, Peroxidase, and Iodinase Activity in Sheep Thyroid Tissue, *Leslie J. DeGroot and Ann Davis.*
17. The Relationship Between Human Serum Ribonuclease Activity and Thyroid Function, *Robert D. Leeper and Rulon W. Rawson.*
18. A Photo-Activated Effect of Flavin Mononucleotide on the Degradation of Thyroxine, *Valerie Anne Galton and Sidney H. Ingbar.*
19. Differential Effects of Radiation on Thyroid Tissue Slices, *J. B. Stanbury, J. Barzelatto and I. P. C. Murray.*

20. Relation Between Electrolyte Metabolism and Functional State in the Thyroid Gland, *Dixon M. Woodbury, Ralph Karler and George R. Heninger.*
21. Effects of Thyrotropin on Thyroidal Water and Electrolytes in the Chick, *David H. Solomon.*
22. Thyroid Stimulating and Exophthalmos Producing Activity of Biochemically Altered Thyrotropin, *Thomas P. Haynie, Richard J. Winzler, Josip Matovinovic, Edward A. Carr, Jr. and William H. Beierwaltes.*
23. Metabolic Balance Studies in Patients with Exogenous Obesity on Weight Reduction Diets and on Maintenance Diets Plus D-L-Triiodothyronine, *K. R. Crispell, Gerald Williams and William Parson.*

Friday, May 5—2:00 p.m.

24. Presidential Address: *Alexander Albert*
25. Van Meter Prize Essay
26. Radioiodine in the Diagnosis and Therapy of Hyperthyroidism Caused by Hyperfunctioning Adenoma, *Penn G. Skillern, Marvin Clamen and E. Perry McCullagh.*
27. Round Table Discussion on the Treatment of the Non-Toxic Nodular Goiter.
    Moderator: *Rulon W. Rawson.*
    Participants: *Warren H. Cole,*
    Lawrence W. Sloan,
    Lewis B. Woolner,
    Monte A. Greer,
    William H. Beierwaltes.

BUSINESS MEETING

Saturday, May 6—8:30 a.m.

28. Measurement of the Iodide Uptake of the Thyroid Gland by Neutron Activation Analysis, *Henry N. Wagner, Jr., Wil B. Nelp and Lt. James H. Dowling.*
29. Differences in the Thyroidal Response of Euthyroid and Hyperthyroid Patients to Antithyroid Substances, *M. L. Mitchell, M. E. O'Rourke and A. B. Harden.*
30. Electroencephalographic Changes Accompanying Thyroid Deficiency in Man, *Robert W. Lansing, and J. B. Trunnell.*
31. Preoperative Use of Reserpine in Hyperthyroidism, *I. Darin Puppel.*
32. Thyroid Carcinoma, *Robert N. Hirabayashi and Stuart Lindsay.*
33. The Effectiveness of Treatment of Thyroid Carcinoma, *J. A. Buckwalter, R. E. Soper, J. S. Medaras and E. E. Mason.*
34. The Estimation of I^{131} Dose in Thyroid Cancer, *Richard S. Benua, Norma R. Cicale, Martin Sonenberg, and Rulon W. Rawson.*
35. Metastatic Neoplasms of the Thyroid Gland, *K. Shimaoka and J. E. Sokal.*

Symposium on Hypertension: Recent Advances

This Symposium will be held under the auspices of Hahnemann Medical College and Hospital, Philadelphia, *May 4–7, 1961.* Included are sessions on endocrine aspects, catecholamine metabolism, and drugs that affect catecholamine metabolism. For further information, write to the Program Director, Dr. Albert N. Brest, Hahnemann Medical College and Hospital 230 Broad Street, Philadelphia 2, Pennsylvania.